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Extended-spectrum β-lactamase–producing *Gram negative Bacteria* from Gowns and Mobile phones of Tikur Anbesa Specialized Hospital Health care workers

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This is to certify that the research thesis prepared by **Shambel Araya Haile**, entitled with; *Extended Spectrum Beta lactamase producing Gram negative bacteria isolated from Gowns and Mobile phones of Tikur Anbesa Specialized Hospital Health care workers,* in partial fulfillment of the requirement for the degree of master of sciences in **Medical Microbiology** complies with the regulation of the University and meets the accepted standards with respect to quality.

Signed by the examining committee:

Internal examiner……..Signature……. Date……..

External examiner……..Signature……. Date…….

Advisor……..Signature……. Date…….

Advisor……..Signature……. Date…….

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Chairman of department

Table of Contents

List of Tables

List of Figures

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First of all, I would like to thank to our almighty GOD who endowing my good health throughout my life. Then I would like to thank Addis Ababa university department of Medical Microbiology, Immunology and Parasitology for accepting to do the research project and overall MSc programme.

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Abbreviations

AST……...Antimicrobial susceptibility tests

CLSI …….Clinical and Laboratory Standards Institute

CONS……..…..Coagulase negative *staphylococcus*

CI…….…….Confidence interval

ESBL……Extended spectrum β- lactamase

HAI……...Hospital acquired infection

HCAI……Health care associated infection

HCWs……..……Health care workers

ICU…….…..Intensive care unit

KMP……..Keypad mobile phones

MDR……Multi drug resistance

MP……Mobile phone

MRSA……..Methicillin resistant *S.aureus*

MSSA…….…….Methicillin susceptible S. *aureus*

PI……...……Principal investigator

SPSS…….…...Statistical package software for social science

TMP……Touch screen mobile phone

TASH……..…Tikur Anbesa Specialized Hospital

VRE……. Vancomycin resistant *Enterococci*

Abstract

Background: Extended spectrum β-lactamases are rapidly evolving group of enzymes produced by certain bacteria that are able to hydrolyze cephalosporin, penicillin and monobactam. Mobile phones and gowns serve as reservoir of ESBL producing microorganisms that could be easily transmitted to the health care workers hands from. Hospital-acquired infection is an increasing global concern for patient safety and it affects more than 25% of the total health care admissions in developing countries.

Objective: The aim of the study was to determine the burden of extended spectrum β- lactamase producing gram negative bacteria and assess their antimicrobial susceptibility patterns isolated from mobile phones and gowns of health care workers at Tikur Anbesa specialized hospital.

Methods: A laboratory based cross sectional study was done from January 2019 to April 2019. A total of 572 paired samples were collected from mobiles and gowns. Bacterial isolates were screened for ESBL production using CHROME agar ESBL and confirmed using cefotaxime, cefotaxime-clavulnic acid and ceftazidime, ceftazidime –clavulnic acid method according to clinical laboratories standard institutes.

Results: Overall, the magnitude of gram negative bacteria isolated from mobile phones and gowns was 454/572(79.4%) and 477(83.4%) respectively, and out of 454 gram negative bacteria isolated from Mobile phones, 48/572 (8.3%) were extended spectrum β-lactamases producers. About 54/572(9.4%) health care workers gowns were also contaminated with extended spectrum β-lactamases producing bacteria. *K .pneumoniae, Acinetobacter, E.coli* and *Citrobacter* were the most dominant isolates. ESBL producers were highly resistant to ampicillin (81%-100%), cotrimoxazole (67%-100%) and chloramphenicol (67-100%) and highly sensitive to meropenem (96%-100%), imipenem996-100%) and piperacillin-tazobactam(90%-100%).

Conclusion: Health care workers mobile phones and gowns were contaminated with pathogenic bacteria. Since gowns and mobile phones could serve as a vehicle and a reservoir for pathogenic microorganisms, health care workers should be aware on the public health risk of hospital-acquired infection and appropriate interventions mechanisms should be in place to reduce the burden and cross transmission.

Key words: Extended spectrum β- lactamase, gram negative bacteria, health care workers, mobile phone and gown

1. Background

1.1 Introduction

Extended spectrum β-lactamases (ESBLs) are defined as enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin and penicillin groups. They are therefore resistant against beta-lactam antibiotics like ceftazidime, ceftriaxone, cefotaxime and oxyimino mono bactam. Carbapenems and cephamycines are effective against ESBL producing bacteria. Generally, ESBLs are inhibited by clavulnic acid and Tazobactam in combination with other class of antibiotics. ESBLs are found in gram-negative bacteria, especially in Enterobacteriaceae [1, 2]. The most important β-lactamase that is prevalent is TEM-1. It is estimated that more than 90% of ampicillin resistance among *E.coli* is related to the presence of TEM-1. TEM-1 is able to hydrolyze penicillin and first generation cephalosporin. The first derivative of TEM-1 is TEM-2, with a single replacement of amino acids [3, 4]. The difference between β lactamase enzymes is the substitution of amino acids that produces different phenotype of enzymes. Another prevalent type of β- lactamase is SHV-1, which was described initially in *K. pneumoniae* [4, 5]. It is estimated that SHV-1 is responsible for plasmid mediated ampicillin resistance in bacteria which harbors it. There are also other less common β-lactamases like CTX-M beta lactamase, OXA β-lactamase and other plasmid-mediated ESBLs, such as PER, VEB, GES, and IBC β-lactamases have been described but are uncommon and have been found mainly in *P. aeruginosa* and at a limited number of geographic sites. The replacement of amino acids causes the change in enzyme structures and its activities [6-8]. ESBL-producing Gram negative bacteria like *Acinetobacte*r, *Pseudomonas* and various Enterobacteriaceae (including *Klebsiella,* E. *coli*, *Serratia,* and *Proteus*) are of major concerns due to concomitant multidrug resistance and causes of severe and often deadly infections such as bloodstream infections and pneumonia [9].

Beta-lactam antibiotics are the major bulk of prescribed antibiotics across the globe because of their efficacy, broad spectra and low toxicity. However, a misuse and due to natural resistance mechanism of bacteria to these antibiotics has emerged in the development and spread of drug resistant bacterial pathogens especially in the developing countries [10]. Of particular concern is the increased occurrence of gram negative bacteria, including multidrug resistant non fermenters

(*Acinetobacter baumannii* and Pseudomonas species) and Enterobacteriaceae producing extended spectrum β-lactamase (ESBL) and carbapenemases in severe healthcare-associated infections as a significant clinical threat for medical community in recent decades. The broad substrate profile of these enzymes may affect entire β- lactam agents, and also the organisms with these enzymes are additionally found resistant to aminoglycosides and fluoroquinolone, further compromising the therapeutic choices for severe infections in Intensive care unit (ICU)[11, 12].

Healthcare-associated infections (HAIs) are associated with significant mortality, morbidity, and high economic burden and they are a major challenge to the healthcare system. The constant handling of MPs by users in hospitals (by patients, visitors and health care workers (HCWs), etc…) makes it an open breeding place for transmission of microorganisms, as well as health care-associated infections (HCAIs). This is especially so with those associated with the skin due to the moisture and optimum temperature of human body especially our palms [13].

The use of cell-phone is increasingly widespread in health centers, and even reaching operating rooms. Furthermore, it has been shown that since mobile phones (MPs) may serve as reservoir of microorganism that could be easily transmitted from MPs to the HCWs hands and therefore facilitate the transmission of bacteria from one patient to another in different hospital communities [14].

Gowns have been used for years in hospital settings to reduce cross-transmission and the risk of disease acquisition by HCWs. White coats are worn primarily for identification, but there has always been some concern that white coats, like nurses uniforms and other hospital garments, may play a part in transmitting pathogenic bacteria in a hospital setting, as white coats are known to be potentially contaminated with pathogenic drug resistant bacteria [15, 16].

Although they play an important role in infection prevention and control; textile materials and personal protective equipment used in health care settings are known to be one of the sources of cross-infection. Gowns are recommended to prevent transmission of infectious diseases in certain settings; however, laboratory and field studies have produced mixed results of their efficacy [16].

1.2 Statement of the Problem

Not all that so long ago, hospitals relied on pen and paper for measuring, monitoring and reporting information. Today, handheld computers and mobile devices have taken their rightful place in hospitals around the world. On a daily basis, clinicians use these powerful devices to gain instant access to a wealth of mission critical information from real-time lab results to a change in a patient's condition. Patient care and clinical outcomes have dramatically improved. Along with the many important benefits these devices provide, however, come new concerns about infection control and the need to keep the devices cleaned and disinfected.

Although significant progress has been made in preventing some healthcare-associated infection types, there is much more work to be done. On any given day, about one in 31 hospital patients has at least one healthcare-associated infection in USA [17]. Hospital-acquired infection (HAI) is an increasing global concern for patient safety. It affects more than 25% of the total health care admissions in developing countries [17, 18]

In the last decade, cell phone use has penetrated clinical practice, providing rapid access to medical information and allowing efficient communication with colleagues worldwide. However, cell phone use in sensitive settings and lack of disinfection, coupled with their portability, makes them a potential source of infection [19]. Evidence of mobile phone contamination at hospitals has been observed; these may be implicated in outbreaks at ICUs. Therefore, cell phones probably represent a constant infection risk for patients, and developing countries are likely at greater risk [14, 19]

In this mobile era, the increased use of mobile phone in hospitals wards may have more serious hygiene consequences, because unlike fixed phones, mobile phones are often used in these areas close to the patients and these patients are more vulnerable to HAIs [13, 14].

Gowns have been shown to harbor potential contaminants and may have a role in the nosocomial transmission of pathogenic microorganisms [20, 21].

Many HCWs wear their white coats in the non-clinical and non-practical classes, library, Cafeteria and in the resting areas around their hospitals. It is not uncommon to see white coats

being carried on hospital areas or being carried around outside the hospital premises. So, gowns serve as a means of infection transmission in the hospital community [21].

ESBLs are spread via direct and indirect contact with colonized/infected patients and contaminated environmental surfaces. ESBLs are most commonly spread via unwashed hands of health care providers after handling of their patients. Risk factor for ESBL-producing bacterial acquisition includes;

1. Direct transfer from another hospital, nursing home and retirement home.

2. Any hospital, nursing retirement home or other health care facility admission in the past year,

3. Patients receiving home health care services or hemodialysis

4. Patients living in a communal living setting (e.g. shelter, halfway house).

5. Patient who previously had an antibiotic-resistant organism (e.g., MRSA, VRE).

6. Patient-to-patient transmission of ESBL-E via the hands of healthcare workers appeared to be common. Therefore, concomitant outbreaks can occur in a nosocomial setting in which most patients carry or display contamination with ESBL producing bacteria [22,23].

Hand washing may not usually be performed often enough and many people use personal mobile phone in the course of a working day and the potential role of mobile phones as a source of microbial transmission is considerable. People rarely disinfect mobile phones and they are cumbersome to clean. As a result, these devices have the potential for contamination with various bacterial agents [13]. Little work has been reported in our country on bacterial contamination on cell phones used by HCWs [24, 25]. But many researchers have studied cell phone contamination among healthcare workers and in the community [26-30]. However, little work has been reported in Ethiopia on bacterial contamination on gowns and mobile phones used by HCWs and there is no published data on ESBL rate from both mobile phones and gowns of HCWs in our setting. So, the present study aimed to fill this gap.

1.3 Significance of the study

Healthcare associated infections (HCAI) and antimicrobial resistance are principal threats to the patients of intensive care units and are the major determining factors for patient outcome. Objects with frequent hand contact such as mobile phones can serve as reservoirs from which infections can spread to the hands of healthcare providers and then to patients. These mobile devices are also carried out of the hospital and to the home of HCWs and hence to the community. Therefore, approaches to counter HCAI should not only include encouraging hand hygiene but also cater to the eradication of all the factors that might play a role as a reservoir of infectious agents.

The role of bacterial contamination of uniforms of health care workers in the horizontal transmission of bacteria is very high. Contaminated uniforms acted as vectors for the continued dissemination of bacteria from patients to HCWs and vice versa

Understanding sources of these infections will help hospital authorities and services improve information dissemination as well as adopt more pragmatic approach in helping reduce such infection and to formulate full guidelines about restricting the use of cell phones in clinical environments, hand hygiene, and frequent decontamination of mobile devices and their gowns to limit the risk of cross contamination and healthcare-associated infections caused by bacteria carried on inanimate objects..

2. Literature Review

Increased use of beta-lactam antibiotics has been associated with the emergence of β lactamase mediated bacterial resistance. Beta-lactamases are enzymes that inactivate beta-lactam antibiotics by cleaving the beta-lactam ring through an irreversible hydroxylation of an amide bond [26].

Extended spectrum beta-lactamases are beta-lactamase enzymes that have the ability to hydrolyze penicillins, extended-spectrum Cephalosporins (oxyimino-beta-lactams), and aztreonam, but not carbapenem or cephamycin antibiotics. About 136 ESBLs are reported worldwide among different bacterial species, including Enterobacteriaceae and non-fermentative gram negative bacteria such as *Pseudomonas* and *Acinetobacter Spp* [27].

In a study conducted by Sedighi I *et al*; in Iran for the detection of microbial contamination rate in mobile phones of health workers among 125 clinical staffs, 15 (12.0%) physicians, 10 (8.0%) residents, 31 (24.8%) interns, and 69 (55.2%) nurses. 99.2% of mobile phones in clinical staff group demonstrated growth of bacterial species. The most common isolated bacteria were consecutively coagulase-negative Staphylococci (82.4%), *S. aureus* (20.0%), *Pseudomonas* species (12.0%) and *Salmonella paratyphi* (10%). Antimicrobial susceptibility tests for the isolates coagulase-negative *Staphylococci*; the highest resistance rate was detected for oxacillin. In addition, *S. aureus*, the highest resistance was detected for oxacillin. However, for *Pseudomonas Spp*, considerable resistance rate to ampicillin was detected [28].

A cross sectional study conducted by Loyola *et al.,in P*eru to assess microbial contamination rate in mobile phone of HCWs. A total of 105 Enterobacteriaceae were isolated; the most common isolates were 48 (45.7%) *Enterobacter spp*, 34 (32.4%) E coli, 13 (12.4%) *K. pneumoniae*, and 10 (9.5%) *K.oxytoca*. A third of the isolates were ESBL producers (35/105, 33.3%), and ESBL production was found in all types of Enterobacteriaceae (P = .004), but it was predominately higher in *E. coli* (55.9%) and *K.pneumoniae* (30.8%).Nearly half of all bacteria isolated were multidrug resistant (MDR; 56/105, 53.3*%). K.oxytoca* strains were marginally less MDR than the other Enterobacteriaceae isolated (20.0% vs 52.1%–64.7%, respectively; P = .099). Also, *Enterobacter spp* and *K. Oxytoca* presented lower levels of ESBL (18.8%–30.0%) than *K. pneumoniae* and *E .coli* (30.8%–55.9%, P < .005). Tobramycin, gentamicin, Trimethoprim- sulphamethoxazole, and ciprofloxacin resistance

were significantly associated with ESBL production in all isolates. Additionally, nearly all of the isolates (99/105, 94.3%) were susceptible to amikacin, and 71.4% (50/70) of non-ESBL isolates were susceptible to all drugs tested in this study. Two ESBL-producing strains, one *E.coli* and one *K. pneumoniae*, were resistant to all of the drugs tested. Four other ESBL-producing strains, three *Enterobacter spp* and one *E. coli*, were resistant to all but 1 of the drug families tested (carbapenem). No resistances to carbapenem group were found by phenotypic methods [29].

A study carried out to investigate the prevalence of multidrug resistant producing pathogens contaminated mobile phones (MPs) high prevalence of multidrug-resistant isolates reaching (71.8%), whereas Extended-spectrum β-lactamases (ESBLs) producing *K. pneumoniae*, *E. coli* and MRSA accounted (27.6%) of the isolates. *E. cloacae* (88.9%) and *S. aureus* (85.7%) were found to be the principle MDR isolates. Likewise 76% of *A. baumannii, K. pneumonia* and *E. coli* were MDR. High prevalence rate of resistant patterns were observed among Enterobacteriaceae strains with exception *of E. agglomerans* to ampicillin, cephalosporin and fluoroquinolone. Better activity was limited to amikacin and Tigecycline, which elicited the highest susceptibility levels. Resistance rates toward carbapenems among *A. baumanii* and *P. aeruginosa* were 42.8% and 33.4%respectively [30].

Another study conducted by Ustun *et al*; among183 mobile phone: In total, 179 (97.8%) culture-positive specimens were isolated from the 183 mobile phones, including 17 (9.5%) MRSA and 20 (11.2%) ESBL -producing *E.coli*. No statistical difference was observed in the recovery of MRSA ($p = 0.3$) and ESBL-producing *E. coli* ($p = 0.6$) between the HCW groups. Forty-four (24.6%) of the 179 specimens were isolated from mobile phones of ICU workers, including two MRSA and nine ESBL-producing *E. coli*. HCWs mobile phones are potential vectors for transferring nosocomial pathogens between HCWs, patients, and the community [31].

Selim *et al*(2015); had also conducted a study in Egypt. This work revealed that the majority of isolated bacterial contaminants were mixed with more than one organism. It has been found that all mobile phones tested from the laboratory (100%) yielded mixed organisms, followed by 90% from dialysis unit and 70% from triage area. As regards isolated organisms in this study, MRSA was detected in 53% of the samples, followed by CoNS (50%), *Bacillus* (43%), *Diphtheroids*

(30%), methicillin-susceptible *S.aureus* (MSSA) (18%), *E. coli* and *V. streptococci* (13% each), Micrococci (10%), *K. pneumoniae* and ESBL *K. pneumoniae* (8% each). The least encountered isolates were *A. baumanii* and Candida (3% each). In this study, *E. coli* represented 10%. Three ESBL *Klebsiella spp* were isolated. Two of the three isolates were revealed from laboratory workers mobile phones and one from a nurse cell phone. On the other hand, the one *A. baumanii* strain encountered in this study was isolated from the mobile phone of a laboratory technician and was found to be multi drug resistant [32].

In Ethiopia a study conducted by Gashaw *et al*; in Gondar made an isolation rate of 98.3%. On this study Only two mobile phones had showed contamination with multiple bacterial species. A total of 59 bacterial isolates were identified from these mobile phones. From the isolates gram-positive bacteria accounted for 77.9%, CONS being the most frequently (47.5%) isolated bacteria followed by *S. aureus* (27.1%) and *S. pyogenes* (3.4%). *E. coli* (6.8%) was the most frequently isolated Gram-negative bacteria followed by *P. stuartii* (5%). *E. cloacae*, *K. pneumoniae*, and *Citrobacter species* each accounted for 3.4% of the isolates. Among the gram-negative bacteria *E. coli* were found 100% sensitive for ciprofloxacin, gentamycin, and trimethoprim-sulphamethoxazole. On the other hand, all isolates of *E. cloacae* were 100% resistant for ceftriaxone, ciprofloxacin, amoxicillin, and chloramphenicol. Multiple antimicrobial resistances in this study ranged from resistance for two drugs up to resistance for six drugs. Accordingly, 10 isolates (16.9%) were resistant to two drugs and 1 isolate (1.7%) was resistant to six drugs [24].

Another cross sectional study conducted by Misgana *et al* in Jimma town; bacteriological analysis of swab samples from the mobile phones showed that 86.37% (57/66) of HCWs mobile phones had evidence of bacterial contamination. Few gram negative bacteria, that is one *K. pneumoniae* and one *Serratia Spp*, were isolated from HCWs mobile phones and were resistant to all the beta-lactam antimicrobials used including cefotaxime [25].

A cross sectional study conducted by Treakle *et al.* in Maryland, United States included 149 participants who were wearing their white coats at study entry, out of which 109 attended medical grand rounds and 40 attended surgical grand rounds. Overall, 22.8% (95% CI = 16.1%

to 29.6%) were contaminated with *S. aureus* and 4% (95% CI = 0.8% to 7.1%) were contaminated with MRSA. Twenty-nine white coats of the internal medicine participants (26.6%; 95% CI = 18.3% to 34.9%) and 5 coats of the surgery participants (12.5%; 95% CI = 2.3% to 22.7%) were found to be contaminated with *S. aureus*. Six white coats of the internal medicine participants (5.5%; 95% CI = 1.2% to 9.8%) and no white coats of the surgery participants were contaminated with MRSA. The most common reasons given for wearing white coats were for "professionalism" and "to hold things" [33].

A study by Qaday J *etal;* in Tanzania on white coats of health care workers revealed that among 180 white gowns; One hundred and thirty two (73.33%) out of 180 whites coats were contaminated with different pathogens. The most dominant ones were *S. aureus*, 120 (91.67%), *Pseudomonas aeruginosa*, 9 (6.82%), and *E. coli*, 3 (2.27%). Most of the white coats in this study were used less than 3 days before another round of washing (132/180) (73.33%) [34].

In a cross sectional study which was conducted by Akanbi ii A.A *et al*; in Nigeria; a sum of 80 doctors white coat was found contaminated; with the Resident doctors' white coats carrying the highest number of 52, representing 65% of the total number of white coats contaminated. The degree of contamination by sex across the cadre, 74 of which were males and 29 females; representing 71.8% and 28.2% of the total population respectively. A sum of 57 males representing 71.2% of the total population of the doctors' white coats contaminated. The degree of contamination by department, 35 of which were drawn from surgery, 29 from medicine, 20 from pediatrics and 19 from obstetrics and gynecology: representing 34.0%, 28.2%, 19.4% and 18.4% of the total population respectively. This study revealed that 77.7% of the coats were contaminated with Staphylococcus aureus (45.1%), *Staphylococcus epidermidis* (26.2%), *K. pneumoniae* (22.6%), *P. aeruginosa* (3.7%) and *Enterococcus faecalis* (2.4%). White coats of male resident doctors were more contaminated than that of the female resident doctors, while white coats of doctors from the department of surgery had the highest degree of contamination than other departments [35].

3. Objectives

3.1 General objective

- To determine the burden and susceptibility pattern of Extended-spectrum β-lactamase producing gram negative bacteria from mobile phones and gowns of health care workers in Tikur Anbesa Specialized Hospital.

3.2 Specific objectives

- To determine gram negative bacteria contamination rate in mobile phone and gown of Tikur Anbesa specialized hospital HCWs.
- To determine the rate of ESBL producing gram negative bacteria in mobile phone and gown of Tikur Anbesa specialized hospital HCWs.
- To assess antimicrobial susceptibility pattern of gram negative bacteria isolates from mobile phone and gown of HCWs to commonly used antibiotics.

4. Materials and methods

4.1 Study design

A laboratory based cross-sectional study was conducted in Tikur Anbesa Specialized Hospital to isolate ESBL producing bacteria and their antibiotic susceptibility pattern. Stored isolates from an ongoing PhD project (PhD Candidate: Kassu Desta, Title of project: Burden of Methicillin Resistant *Staphylococcus aureus* (MRSA) and associated factors at TASH (evidence from colonization of patients, health care workers, administrative staffs and selected inanimate objects) was used for performing the identification and Antimicrobial susceptibility patterns of gram negative bacteria.

4.2 Study Period

The study was conducted from January 2019 to April 2019

4.3 Study area

This study was done at Tikur Anbesa Specialized Hospital (TASH) which is located at Zambia St, Addis Ababa, Ethiopia. The Hospital is a teaching hospital of Addis Ababa University, College of health Science. With the opening of the Tikur Anbesa Specialized Hospital (TASH) in 1972, the hospital became the only site for training medical doctors .It is Ethiopia's largest tertiary teaching and referral hospital admitting and treating different patients under various specialties, with over 800 beds, and serves as a training center for undergraduate and postgraduate medical students, dentists, nurses, midwives, pharmacists, medical laboratory technologists, radiology technologists, and others who shoulder the health problems of the community and the country at large. Tikur Anbesa specialized Hospital is now the main teaching hospital for both clinical and preclinical training of most disciplines. It is also an institution where specialized clinical services that are not available in other public or private institutions are rendered to the whole nation.

4.4 Source population

The source population for this study was Hospital health care workers in TASH and bacterial isolates from mobile phones and gowns of HCWs.

4.5 Study population

The study populations for the project are those HCWs working in TASH and provided swabs from their mobile phones and gowns

4.6 Inclusion and exclusion Criteria

4.6.1 Inclusion criteria

- ✓ Gram negative bacterial isolates available in storage.
- ✓ Data availability of socio demographic factors.

4.7 variables

4.7.1Dependent Variables

- ✓ ESBL producing gram negative bacteria
- ✓ Burden of gram negative bacteria
- ✓ Antimicrobial susceptibility pattern

4.7.2 Independent Variables

- ✓ Age
- ✓ Sex
- ✓ Profession
- ✓ Mobile type
- ✓ Gown type
- ✓ Service year of HCW's
- ✓ Frequency of Gown changing

4.8 Sample size

4.8.1 Sample size determination

We took a total of 572 paired samples collected from June 2018 to September 2018 by the an ongoing PhD project purposively and generally we detected ESBL production from a total of 572 paired samples for both mobile and gown each and taking 50% from the total isolates we conducted further characterization of NON ESBL producer gram negative bacteria for both mobile phones and gowns of health care workers was done

4.9 Sampling Technique

HCWs were randomly selected and proportionally allocated from the following hospital units; from the Medical laboratory personnel (156), Pharmacist (28), Nurses (293), Medical Doctors (156) and others HCWs (59) in the ongoing PhD project, from which we obtained the stored isolates. All isolates were screened for ESBL production. Through systematic random sampling technique from the pre collected and stored 572 samples from mobile and gowns of HCWs 557 isolates (277 for mobile and 280 for gown) were further processed in the current study.

4.10. Data collection and laboratory processes

4.10.1. Sample collection

The stored isolates characterized in the current study were from samples from mobile phones and gowns of TASH health care workers which were collected using a sterile cotton swab moistened with sterile normal saline that was rolled over a measured area of exposed outer surfaces of the mobile phones. Included were the most frequent areas of contact with the fingers: the buttons of the keypad, earpiece, back side and lateral side of the mobile phone .Samples from gown was also collected in similar way with that of mobile and the most included areas were areas which are most exposed to contamination such as around the pockets and other surface areas. We also took the socio demographic data of dependent and independent variables of the participants from a pre collected questionnaire.

4.10.2. Laboratory procedures.

4.10.2.1. Bacterial identification

Isolation of gram negative bacteria was performed by a surface streak procedure on MacConkey agar (Oxoid Ltd. BashingstoreHampaire, UK) using calibrated loops and incubated aerobically at 37 ^{0}C for 24 hours, cultures which did not grow any colonies at the end of 24 hours incubations were further incubated for additional24 hours. Identification of all the isolates was done using biochemical tests, namely indole, citrate, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, Malonate, catalase and mannitol fermentation. Oxidase test was used for differentiation of *Pseudomonas* and *Acinetobacte*r *Spp.*

The isolates were screened for extended spectrum beta-lactamase (ESBL) production using the ESBL CHROME agar after streaking the stored isolates onto ESBLCHROME agar. Bacterial colonies which

have blue colony appearance were suspected as *K.pneumoniae*, and those with pink colony appearance as E.coli. Those which showed whitish colonies were suspected as other ESBL producing gram negative bacteria. ESBL production for suspected colonies by this screening method was confirmed using double disk diffusion method according to CLSI recommendations. Ceftazidime 30 µg, Ceftazidime-Clavulnic acid 30/10 µg, Cefotaxime 30 µg and Cefotaxime-Clavulnic acid 30/10 µg discs were used. A ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs. the zone diameter of the agent when tested alone confirmed phenotypically ESBL producers [36].

4.10.2.2 Antibacterial Susceptibility testing:

Antimicrobial susceptibility of isolates was tested for all bacterial isolates by the disk diffusion method according to 2018 Clinical Laboratory Standards Institute (CLSI) guideline (36). Standard inoculums adjusted to 0.5 McFarland was swabbed on to Muller- Hinton agar (Oxoid Ltd. Bashing store Hampaire, UK); antibiotic discs were dispensed after drying the plate for 3-5 min and incubated at 37^{o}C for 24 hours. The susceptibility of the isolated gram negative bacteria were tested against Imipenem (10 µg), Ampicillin (10 µg), Trimethoprim/Sulphamethoxazole (1.25/1.23 µg), ciprofloxacin (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Piperacilin (100 µg), Amikacin (30 µg), ceftriaxone (30 µg), Piperacilin- Tazobactam (100/10 µg), Gentamicin (30 µg) using the Kirby-Bauer disc-diffusion technique as previously described (36). The combined disks (10 µg of clavulnic acid plus 30 µg of Ceftazidime/Cefotaxime) in parallel with these cephalosporins were used for detection of extended spectrum beta-lactamases (ESBLs). Quality control was assured by concurrent testing with the American Type Culture Collection (ATCC) strains including *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27852 and *K. pneumoniae* (ATCC 700603) [34].

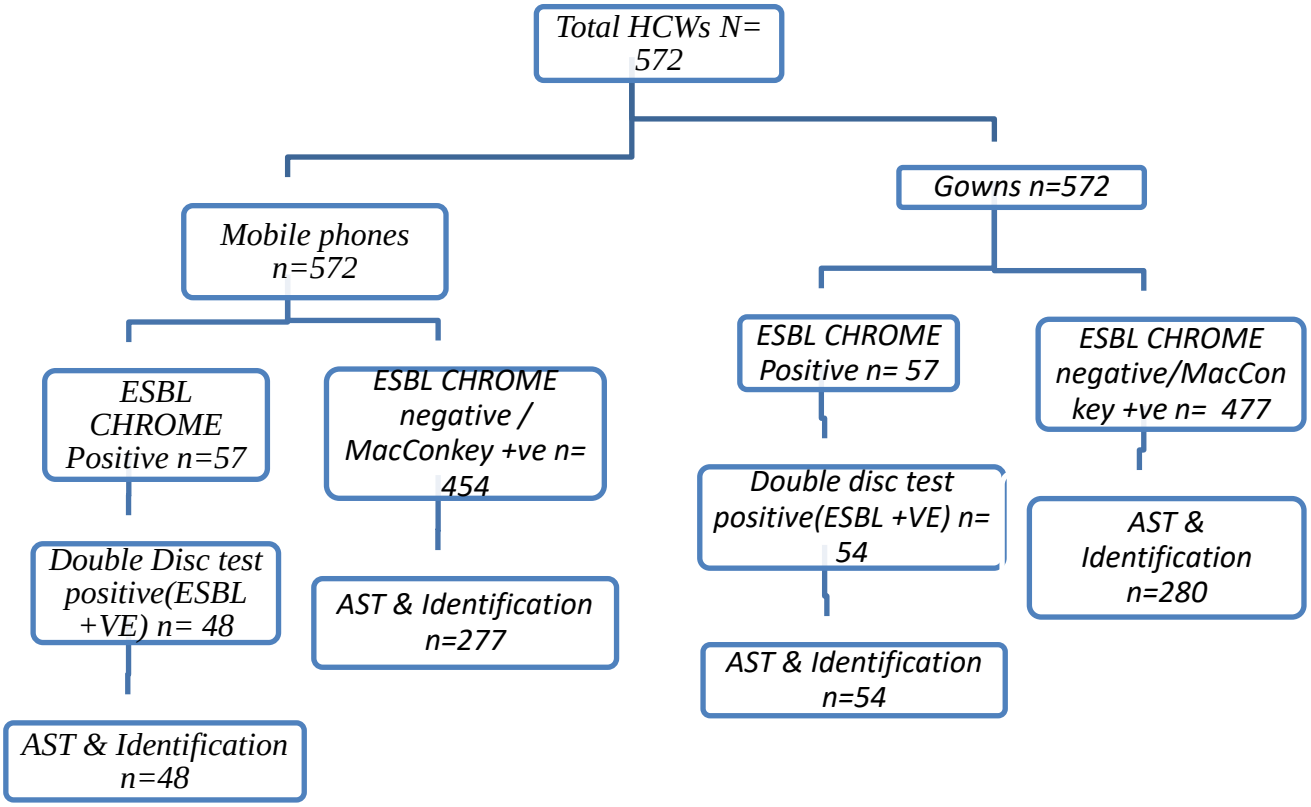

Figure 1: Data collection and laboratory procedure flow chart

4.11. Quality assurance

All the equipment had been checked for proper function. The prepared culture media had been also checked for sterility by incubating the 1-3% percent of prepared media for about overnight and observe for the presence of any growth if there is any growth it was discarded. Its performance had been checked by inoculating known strain of gram negative pathogens in day to day activities. All the laboratory procedures have been performed according to CLSI guide line; all the laboratory procedure activities were performed and proceed after the daily quality control is passed.

4.12. Statistical analyses

Data was collected, double entered, cleaned and analyzed using SPSS version 20 software according to the study objectives. Frequency, percentage, chi-square and bivariate analysis were used for investigation of the outcome. P- Value< 0.05 was used as significantly associated. Categorical data were compared using chi-square test.

4.13. Ethical Consideration

Ethical clearance was obtained from the Department Ethics Review Committee (DREC) of the Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, College of Health Sciences (CHS); Addis Ababa University ,Ethiopia. The samples were taken from an ongoing PhD work which has ethical clearance from the CHS - IRB and National Ethics committee.

5.0 Results

5.1 Socio-demographic characteristics of HCWs

A total of 572 HCWs were included from the different units of the hospital. Of those HCWs 334 (58.4 %) were females. Age of study participants ranged from 20-57 year old and majority (72%) were between the ages of 21-30 years. The mean age of the study participants was 29±6.9. Majority of the participants were nurses in profession (51.2%), followed by Medical Doctors (27.3%). The socio-demographic characteristics, profession and service year of the study participants are shown in Table 1.

Table 1: Socio demographic characteristics of study participants among TASH HCWs, 2019

Variables		**Frequency n=572**	**Percent (%)**
Gender	Male	238	41.6%)
	Female	334	58.4%
Age	20-30	412	72.0%
	31-40	116	20.3%
	41-50	27	4.7%
	>51	17	3.0%
(SD)	29	6.9	
Profession	Medical doctor	156	27.3%
	Nurse	293	51.2%
	Medical Laboratory	36	6.3%
	Pharmacy	28	4.9%
	Other	59	10.3%
Service Year	1-2 year	190	33.2%
	3-4 year	150	26.2%
	5-7 year	112	19.6%
	8-10 year	45	7.9%
	>10 year	75	13.1%

5.2 Overall isolates from mobile phones and gowns

A. Mobile Phones: Total mobile phones sampled in this study were 572 and from these 454 of them was contaminated with at least one gram negative bacteria , total number of isolates found on this study was 538 (374 single,76 double and 4 samples with three isolates). From these 57/572(10%) of them had shown growth on CHROME agar. Two hundred and seventy seven CHROME agar negative isolates (Non ESBL) were also further characterized from the total 538 isolates obtained from 572 samples using systematic random sampling.

B. Gowns : Total number of gowns sampled were 572 and from these 477 of them was contaminated with at least one gram negative bacteria , total number of isolates found from gowns of HCWs were 574 (378 single,95 double and 2 samples with three isolates). Using systematic random sampling 280 isolates from 574 CHROME agar negative isolates (Non ESBL) were also further characterized.

5.3 Associated factors for bacterial contamination in mobile phones of HCWs

The bacteria identified in this study were isolated from a total mobile phones of 572; 156(27.3%) physicians, 293(51.2%) nurses, 36 (6.3%) medical laboratory personnel, 28(4.9%) pharmacy and 59(10.3%) other HCWs. Of these total phones; 465(81.3%) were touch screen mobile phone (TMP) and 107(18.7%) were keypad mobile phones (KMP). Four hundred fifty four mobile phones were contaminated with gram negative bacteria and the rate of contamination was higher in females 275/334(82.3%) than Males (75.2%) and this was significantly associated (p-value= 0.039).

This study revealed that 454 (79.4%) of mobile phones were contaminated with one or more than one gram negative bacteria. The majority of mobile phones 374(82.4%) contaminated with bacteria were found contaminated with single type of colony and 76(16.7%) had two types of colonies and 0.9% (4/454) had three types of colonies but this was not significant (p>0.282) as shown in table 2.

Ninty one point seven percent mobile phones screened from medical laboratory HCWs had shown a growth of at least one gram negative bacteria, followed by 82.4% from Nurses (Table 2). The difference between these results was found to be statistically significant among medical laboratory personnel (p-value ≤0.044).

In relation to years of service of HCWs in the Hospital, those who served 1-2 years showed a greater degree of contamination 139/190(30.6%) followed by those who served for 3-4 years 120/150 (26.4%)

and only 14.1 % of them had served for greater than 10 years. There was a significant association between the service years of HCWs and whether or not mobile phones would be contaminated with at least one gram negative bacteria ($p \leq 0.038$, CI 0.229-0.958) (Table 2).

Table 2: Associated factors of microbial contamination for mobile phones of HCWs

Variables	Contaminated n (%)	Not contaminated n (%)	Bivariate analysis		P-value
			COR	95% CI	
Gender					
Male n=238(41.6)	179(75.2)	59(24.8)			
Female n=334(58.4)	275(82.3)	59(17.7)	0.651	0.43-0.978	0.039
Age(year)					
20-30 n=412	317(76.9)	95(23.1)	0.209	0.027-1.593	0.131
31-40 n=116	100(86.2)	16(13.8)	0.391	0.048-3.152	0.378
41-50 n=27	21(77.7)	6(22.2)	0.219	0.024-2.003	0.179
>51 n=17	16(94.1)	1(5.9)	R		
Profession					
Medical doctor n=156	111 (71.2)	45 (28.8)	0.987	.405-2.403	0.976
Nurse N=293	243(82.9)	50(17.1)	1.944	0.811-4.661	0.136
Medical laboratory n=36	33(91.7)	3(8.3)	4.4	1.044-18.542	0.044
Pharmacy n=28	20(71.4)	8(28.6)			
Other n=59	47(79.7)	12(20.3)	1.567	0.556-4.416	0.396
Phone Type					
KMP n=107	89(83.2)	18(16.8)		0.779-2.354	0.282
TMP n=465	365(78.5)	100(21.5)	R		
Service Year					
1-2 n=190	139(73)	51(27)	0.468	0.229-0.958	0.038
3-4 n=150	120(80)	30(20)	0.688	0.323-1.462	.330

5-7 n=112	94(84)	18(16)	0.898	0.397-2.027	.795
8-10 n=45	37(82)	8(18)	0.795	0.293-2.154	.652
>10 n=75	64(85)	11(15)	R		

Out of the 572 mobile phones 89/107(83.2%) of KMP was contaminated with at least one gram negative bacteria, 17/89(9.1%) of KMP was contaminated with two gram negative bacteria and only 1/89(1.1%) mobile phone was contaminated with three gram negative bacteria. Number of isolates per specimen was high among KMP compared to TMP. Furthermore, there was no significant difference in microbial contamination according to the type of mobile phone (P.≥0.282) as shown in (Fig 1).

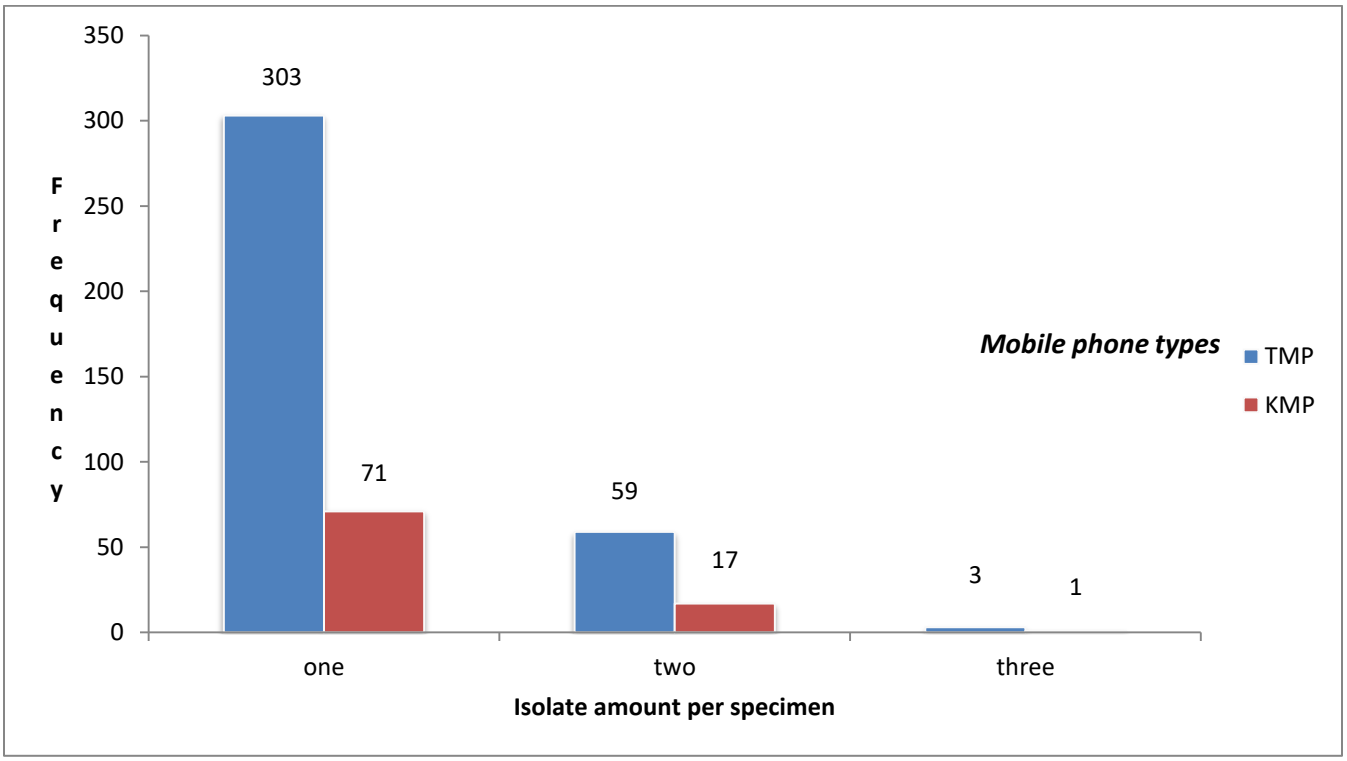

Figure 2: Distribution of bacterial isolates per specimen across different phone types.

5.4 Distribution of ESBL producing gram negative bacteria from mobile phones of HCWs

Out of the total 572 mobile phones screened for ESBL production, 57 (10%) of the isolates were positive for ESBL using screening test method for ESBL production. Among these 48(8.3%) of isolates

were confirmed for ESBL production using confirmatory test method (combination double disk method).

Out of the 48 confirmed ESBL producer gram negative bacteria *K. pneumoniae* was the dominant isolate 13/48 (27.1%) followed by *E.coli* 7/48 (14.6%) and *Acinetobacter Spp* 7/48 (14.6%) (Fig: 2). Other gram-negative bacteria isolated were *K. oxytoca* 6/48 (12.5%), *Citrobacter*spp5/48(10.4%), Proteus vulgaris 4/48 (8.3%) and others.

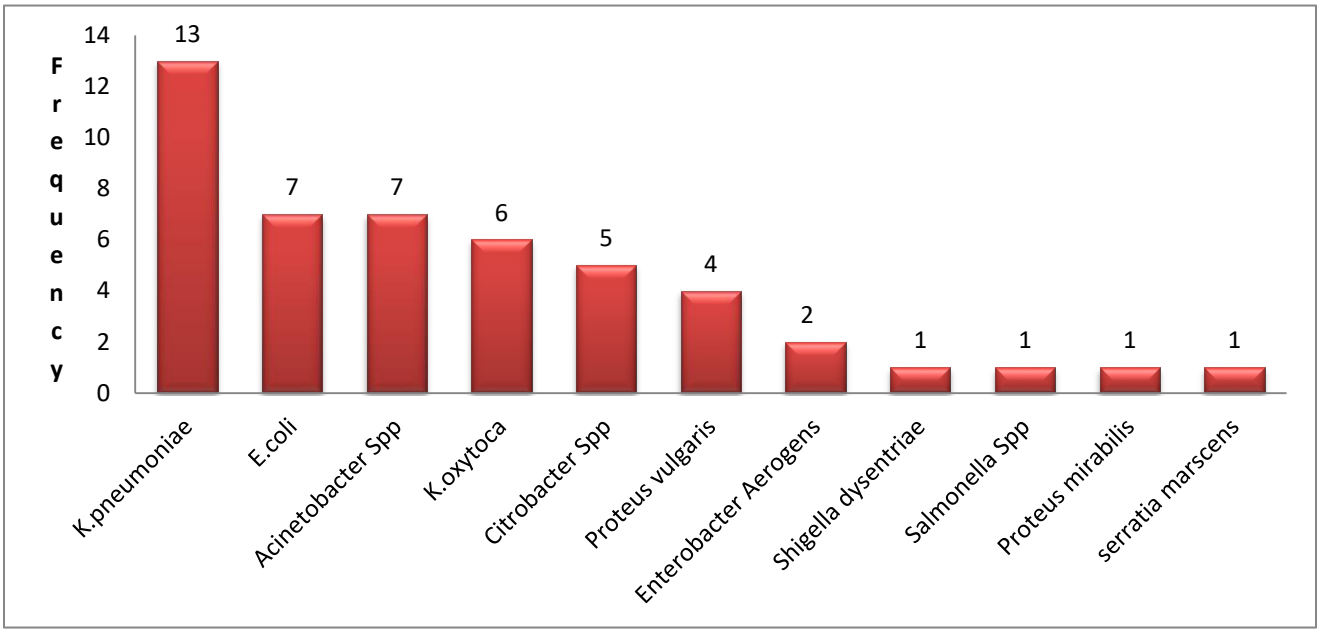

Figure 3: Distribution of ESBL producing Gram negative bacteria from Mobile phones of HCWs

5.5 Antimicrobial susceptibility pattern of ESBL producing gram negative bacteria from mobile phones of HCWs

A total of 48 species were available for antimicrobial testing. Most of these isolates showed widespread resistance to multiple drugs. High-level resistance to ampicillin was observed in 100% of all species except *Citrobacter* (80%) and *K. pneumoniae (*92%). *K. pneumoniae* and *E. coli* also exhibited increased resistance to ampicillin (92%, 100%), chloramphenicol (44%)and cotrimoxazole (28%).Among the other gram negative bacteria, *P.vulgaris* species showed 50% resistance to the ESBL inhibitor piperacilin -tazobactam. However, all isolates were susceptible to MRP, IMP, AMK, CIP and GEN. P. *mirabilis, Salmonella Spp* and *S. dysentriae* also showed 100% resistance each for ESBL inhibitor amoxicillin-clavulnic acid. Resistance to amikacin was found to be comparatively lower in these ESBL

producers' gram negative bacteria (92%-100%). Surprisingly *K. pneumoniae* isolates were resistant for meropenem and imipenem (54%, 62%) respectively (Table 3).

Table 3: AST pattern of ESBL producing gram negative bacteria from mobile phones of HCWs, 2019

Species n=48	**AST**	**Antibacterial agents**													
		AMK	AMP	FOX	CIP	PRL	SXT	C	PZT	TOB	AUG	NOR	GEN	MRP	IMP
E.coli n=7	S	7(100	0	5(72	7(100	7(100	5(72	4(56	7(100	7(100	7(100	7(100	6(86	7(100	6(86
	I	0	0	1(14	0	0	0	0	0	0	0	0	0	0	0
	R	0	7(100	1(14	0	0	2(28	3(44	0	0	0	0	1(14	0	1(14
K. pneumoniae n n=13	S	12(92	1(8	11(85	13(100	11(85	6(46	6(46	12(92	8(62	11(85	13(100	10(77	6(46	5(38
	I	1(8	0	0	0	0	0	3(23	1(8	0	0	0	1(8	0	0
	R	0	12(92	2(15	0	2(15	7(54	4(31	0	5(38	2(15	0	2(15	7(54	8(62
K.oxytoca n=6	S	6(100	0	2(33	5(83	3(50	0	0	6(100	3(50	3(50	4(67	4(67	1(17	2(33
	I	0	0	0	0	0	0	2(33	0	0	0	0	0	0	0
	R	0	6(100	4(67	1(17	3(50	6(100	4(67	0	3(50	3(50	2(33	2(33	5(83	4(67
S.dysentriae n=1	S	1(100	0	0	1(100	1(100	0	0	1(100)	1(100)	0	1(100	1(100	1(100	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	1(100	0	0	1(100	1(100)	0	0	1(100	0	0	0	0
Salmonella Spp n=1	S	0	0	0	0	0	0	0	1(100)	0	0	0	0	0	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(100	1(100	1(100	1(100	1(100	1(100	1(100	0	1(100	1(100	1(100	1(100	1(100	0(100
E.areogenes n=2	S	2(100	0	2(100	2(100	2(100	1(50	0	2(100)	1(50	2(100	2(100	0	2(100	1(50
	I	0	0	0	0	0	0	1(50	0	1(50	0	0	0	0	0
	R	0	2(100	0	0	0	1(50	1(50	0	0	0	0	2(100	0	1(50

Citrobacter Spp n=5	S	5(100	0	3(60	2(40	2(40	0	1(20	4(80)	2(40	3(60	4(80	3(60	3(60	3(60
	I	0	1(20	0	1(20	1(20	0	0	0	0	0	0	0	0	0
	R	0	4(80	2(40	2(40	2(40	5(100	4(80	1(20	3(60	2(40	1(20	2(40	2(40	2(40
P.vulgaris n=4	S	4(100	0	2(50	4(100	4(100	0	2(50	2(50)	4(100	2(50	3(75	4(100	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	4(100	2(50	0	0	4(100	2(50	2(50)	0	2(50	1(25	0	0	0
P.mirabilis n=1	S	1(100	0	0	0	0	0	0	1(100)	1(100	0	0	0	0	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	1(100	1(100	1(100	1(100	1(100	0	0	1(100	1(100	1(100	1(100	0
Acinetobacter SPP n=7	S	7(100	0	7(100	0	0	4(56	5(72	0	0	0	6(86	7(100	0	5(72
	I	0	0	0	0	0	0	0	0	0	0	0	0	1(14	0
	R	0	7(100	0	7(100	7(100	3(44	2(28	7(100	7(100	7(100	1(14	0	6(86	2(28
S.marscens n=1	S	1(100	0	1(100	1(100	1(100	1(100	1(100	1(100)	1(100	1(100	0	1(100	0	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	0	0	0	0	0	0	0	0	1(100	0	1(100	0

Key: S= sensitive, R= Resistance, I= Intermediate, AMP=Ampicillin, AMK= Amikacin, FOX = Cefoxitin, CIP= ciprofloxacin, PRL = Piperacilin, SXT= Trimethoprim – sulphametaxzole (cotrimoxazole), C = Chloramphenicol, TZP = Piperacilin –tazobactam, TOB = Tobramycin , AUG = Augmentin(Amoxicilin- clavulnic acid), NOR = Norfloxacin , GEN = Gentamycin, MRP = Meropenem, IMP, Imipenem

5.5.1Multidrug resistance (MDR) pattern of ESBL producing bacterial isolates from mobile phones

In our study, 79.2% of ESBL-producing isolates showed multidrug resistance to at least one drug from different families of antibiotics (resistance to >3 antibiotics from different classes).Amongst all the bacterial isolates, *K. oxytoca, Salmonella, P. vulgaris and P. mirabilis* showed 100% MDR characteristic, whereas *Klebsiella spp* (77.1%), and *Citrobacter spp* (75%) showed MDR characteristics. Seventy one percent of *Acinetobacter Spp* showed resistance to more than nine tested antibiotics. In *K. oxytoca* 50% of the isolates were resistant to nine and more tested antibiotics; and 16.7% were resistant to three, four and six tested antibiotics consecutively (Table 4).

Table 4: MDR pattern of ESBL producing gram negative bacterial isolates from mobile phones of HCWs, 2019

Isolates n=48	**Antibiogram Resistance n(%)**								
	R1	R2	R3	R4	R5	R6	R7	R8	>R9
E.coli n=7	3(42.9	0	3(42.9	0	1(14.3	0	0	0	0
K.Pneumoniae n=13	3(23.1	0	2(15.4	3(23.1	1(7.7	2(15.4	1(7.7)	0	1(7.7
K.oxytoca n= 6	0	0	1(16.7	1(16.7	0	1(16.7	0	0	3(50
S.dysentria e n=1	0	0	0	0	1(100	0	0	0	0
Salmonella Spp n=1	0	0	0	0	0	0	0	0	1(100
E.aerogens n=2	1(50	0	0	0	0	0	1(50	0	0
Citrobacter Spp n=5	1(20	0	1(20	1(20	0	0	0	0	2(40
P.vulgaris n=4	0	0	0	2(50	1(25	0	0	1(25	0
P.mirabiles n=1	0	0	0	0	0	0	0	0	1(100)
Acinetobacter Spp n=7	0	1(14.3	0	0	0	0	0	1(14.3)	5(71.4
S.marscens n=1	1(100	0	0	0	0	0	0	0	0
Total	9(18.4	1(2.1)	7(14.6	7(14.5)	4(8.3)	3(6.3)	2(4.2	2(4.2	13(27.

Key: Ro = resistance to one antibiotic, R1 = resistance to two antibiotic, R3 = resistance to three antibiotic, …, >R9 = Resistance to more than nine antibiotics.

5.6 Non ESBL producing Gram negative bacteria distribution from mobile phones of HCWS

In addition to identification of ESBL producers, we also tried to study the distribution and AST pattern of non ESBL producers from mobile phones of HCWs. Out of the total 538 suspected gram negative bacterial isolates from572 mobile phones of HCWs 277 bacterial isolates were selected for bacterial identification and antibacterial susceptibility pattern using systematic random sampling method. Out of these 277 bacterial isolates the most predominant gram negative bacteria was *E.coli* accounting 84/277(30.3%) followed by Shigella species 43/277 (15.5%) and *Pseudomonas Spp* 25/277(9.1%) respectively. *S.marscens, Acinetobacter Spp, Morganella morganii* and others which are common causes of hospital-acquired infections were also observed in this study (Fig: 4).

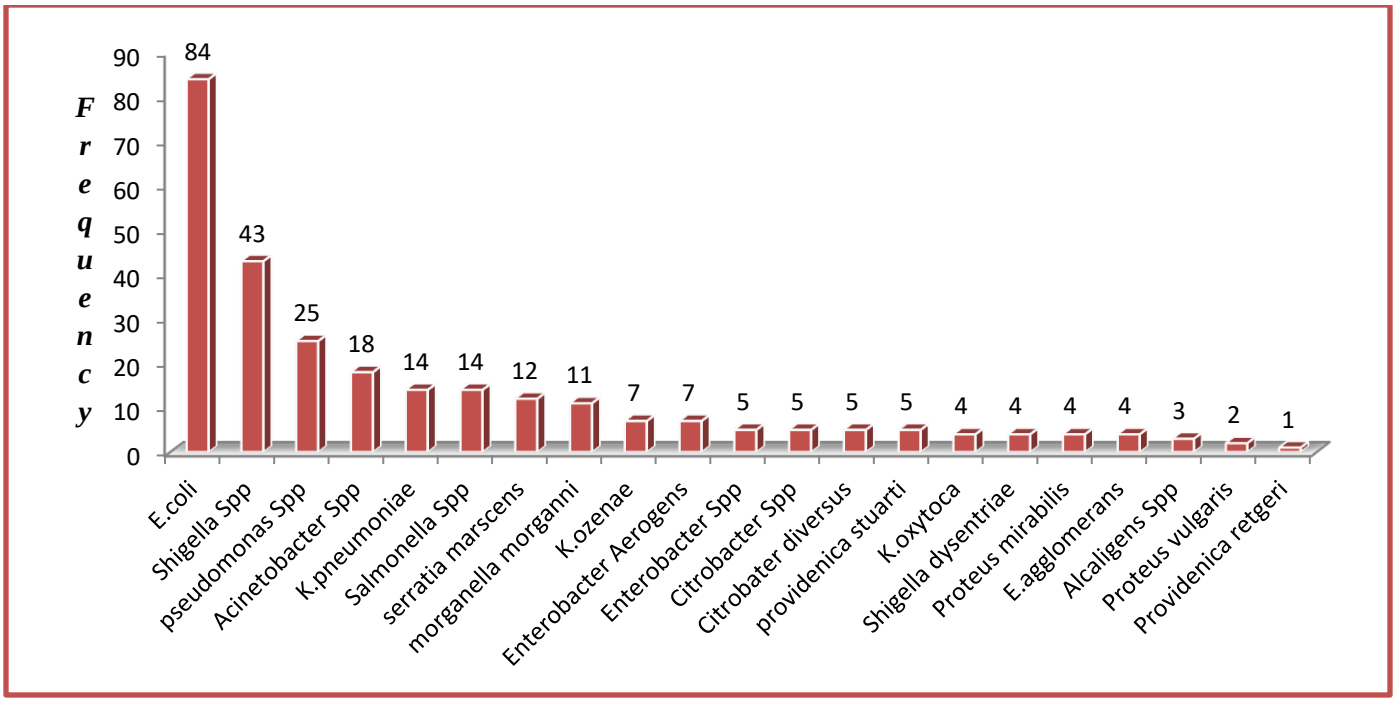

Figure 4: Distribution of Gram negative bacteria isolates from Mobile phones of HCWs

5.7: Antimicrobial susceptibility pattern of gram negative bacterial isolates from mobile phones of HCWs

In this current study, susceptibility pattern of 277 gram negative bacterial isolates towards 14 antibiotics were carried out as recommended by CLSI 2018 (36). After collection of positive culture result about 15 antibiotics were applied in those selected isolates. The predominant gram negative bacteria isolate *E.coli;* showed higher resistance to ampicillin 26/84 (31%), cotrimoxazole & augmentin 15/84(18%) each on the other hand piperacilin- tazobactam83/84 (99%), imipenem 81/84 (96%) and meropenem 79/84 (94%) had also show higher sensitivity. Out of the 43 *Shigella spp* 40(93%) of them were sensitive for meropenem and imipenem; they were also highly resistant (38%) for augmentin followed by ciprofloxacin and norfloxacin 26 % each (Table 5).

Table 5: AST pattern of gram negative bacteria isolates from mobile phones of HCWs

Species	AST	Antibacterial agents n(%)													
		AMK	**AMP**	**FOX**	**CIP**	**PRL**	**SXT**	**C**	**TOB**	**AUG**	**NOR**	**GEN**	**MRP**	**IMP**	**TZB**
E.coli n=84	S	80(95	57(68	64(76	79(94	73(87	69(82	75(89	67(78	69(82	70(83	75(89	79(94	81(96	83(99
	I	0	1(1.2	4(5	0	1(1.2	0	1(1.2	5(6)	0	0	2(2.3	0	0	0
	R	4(5	26(31	16(19	5(6)	10(12	15(18	8(9.8	12(14	15(18	14(16	7(8.3	5(6	3(4	1(1)
K.pneumoniae n=4	S	13(93	9(64.	11(79	9(64	12(86	12(86	13(93	11(79	5(36	8(57	12(86	13(93	14(100	14(100
	I	0	0	1(7	0	0	0	0	0	0	2(14	0	0	0	0
	R	1(7	5(36	2(14	5(36	2(14	2(14	1(7)	3(21	9(64	4(29	2(14	1(7	0	0
K.ozenae n=7	S	7(100	5(72	6(86	6(86	6(86	6(86	7(100)	7(100	6(86	7(100	7(100	7(100	7(100	7(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	2(28	1(14	1(14	1(14	1(14	0	0	1(14	0	0	0	0	0
K.oxytoca n=4	S	4(100	1(25	4(100	4(100	4(100	4(100	4(100)	2(50	1(25	4(75)	4(100	4(100	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	3(75	0	0	0	0	0	2(50	3(75	1(25)	0	0	0	0
Shigella Spp n=7	S	40(93	31(72	37(86	36(84	37(86	38(88	39(90)	38(88	31(72	36(84	38(88	41(95	41(95	42(97.6
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	3(7	12(28	6(14	7(16	6(14	5(12	4(10)	5(22	12(28	7(16	5(12	2(5	2(5	1(3.4
S.dysentriae n=4	S	4(100	1(25	3(75	3(75	3(75	3(75	3(75)	4(100	2(50	4(100	4(100	4(100	4(100	4(100
	I	0	0	1(25	0	0	0	0	0	0	0	0	0	0	0
	R	0	3(75	0	1(25	1(25	1(25	1(25)	0	2(50	0	0	0	0	0
Salmonella Spp n=14	S	12(86	9(63	11(79	14(100	14(100	10(72	11(79)	13(93	13(93	14(100	14(100	14(100	14(100	14(100
	I	0	0	0	0	0	2(14	0	0	0	0	0	0	0	0
	R	2(14	5(37	3(21	0	0	2(14	3(21)	1(7	1(7	0	0	0	0	0

Enterobacter Spp n=5	S	5(100	3(60	2(40	4(80	3(60	5(100	4(60)	4(80	2(40	4(80	5(100	5(100	5(100	5(100
	I	0	0	0	0	0	0	0	0	1(20	0	0	0	0	0
	R	0	2(40	3(60	1(20	2(40	0	2(40)	1(20	2(40	1(20)	0	0	0	0
E.Aerogens n=7	S	6(86	4(57	6(86	6(86	6(86	6(86	7(100)	7(100	3(43	6(86	7(100	7(100	7(100	7(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(14	3(43	1(14	1(14	1(14	1(14	0	0	4(57	1	0	0	0	0
Citrobacter Spp n=5	S	5(100	4(80	3(60	5(100	4(80	5(100	2(40)	1(20	2(40	3(60	4(80	5(100	5(100	5(100
	I	0	0	0	0	0	0	0	2(40	0	2(40	1(20	0	0	0
	R	0	1(20	2(40	0	1(20	0	3(60)	2(40	3(60	0	0	0	0	0
C.diversus n=5	S	5(100	4(80)	5(100	3(60)	3(60)	4(80)	4(80)	5(100	5(100	5(100	4(80)	5(100	5(100	5(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(20)	0	2(40)	2(40)	1(20)	1(20)	0	0	0	1(20)	0	0	0
Pseudomonas Spp n=25	S	22(88	14(58	16(	23(92	24(96	17(67	22(88)	23(92	18(71	21(84	24(96	25(100	23(92	25(100
	I	1(4	1(4	2(8	0	0	0	0	0	0	2(8	0	0	0	0
	R	2(8	10(38	7(29	2(8	1(4	8(33	3(12)	2(8	7(29	2(8	1(4)	0	2(8	0
P.vulgaris n=2	S	1(50	1(50	1(50	2(100	2(100	2(100	2(100)	2(100	0	2(100	2(100)	1(50)	2(100	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(50	1(50	1(50	0	0	0	0	0	2(100	0	0	1(50	0	0
P.mirabilis n=4	S	4(100	2(50	3(75	4(100	3(75	2(50	3(75)	2(50	3(75	4(100	3(75	4(100	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	2(50	1(25	0	1(25	2(50	1(25)	2(50	1(25	0	1(25	0	0	0
Acinetobacter Spp n=18	S	16(88	15(82	16(88	15(83	17	16(88	12(66)	17(94	16(88	14(76	14(76	18(100	17(94	18(100
	I	1(6	1(6	1(6	0	0	0	0	0	0	2(12	0	0	0	0
	R	1(6	2(12	1(6	3(17	1(6	2(12	6(34)	1(6	2(12	2(12	4(24	0	1(6	0

S.marscens n=12	S	12(100	10(83	10(83	11(92	10(83	11(92	10(83)	8(66	10(83	10(83	12(100	12(100	12(100	12(100
	I	0	0	0	0	0	0	0	0	1(8	0	0	0	0	0
	R	0	2(17	2(17	1(8	2(17	1(8	217)	4(33	1(8	2(17	0	0	0	0
P.stuarti n=5	S	5(100	5(100	3(60	5(100	5(100	5(100	5(100	3(60	3(60	5(100	5(100	5(100	5(100	5(100
	I	0	0	0	0	0	0	0	0	1(20	0	0	0	0	0
	R	0	0	2(40	0	0	0	0	2(40	1(20	0	0	0	0	0
P.retgeri n=1	S	1(100	1(100	1(100	0	0	0	1(100	1(100	1(100	0	1(100	1(100	1(100	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	1(100	1(100	1(100	0	0	0	1(100	0	0	0	0
M. morgani n=11	S	9(82	7(64	8(73	9(82	10(91	7(64	9(82)	9(82	10(91	10(82)	10(91	11(100	11(100	11(100
	I	0	0	0	0	0	0	0	0	1(9	0	0	0	0	0
	R	2(18	4(36	3(27	2(18	1(9	4(36	2	2(18	0	2(18)	1(9	0	0	0
Alcaligens Spp n=3	S	3(100	3(100	3(100	3(100	3(100	1(33.3	2(18)	2(67.6	3(100	3(100	3(100	3(100	3(100	3(100
	I	0	0	0	0	0	1(33.3	1(33.3	0	0	0	0	0	0	0
	R	0	0	0	0	0	1(33.3	0	1(33.3	0	0	0	0	0	0
E.agglomeans n=4	S	4(100	3(75	4(100	3(75	3(75	4(100	3(75)	4(100	4(100	3(100	3(75	4(100	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(25	0	1(25	1(25	0	1(25)	0	0	1(25	1(25	0	0	0

Key: S= sensitive, R= Resistance, I= Intermediate, AMP=Ampicillin, AMK= Amikacin, FOX = Cefoxitin, CIP= ciprofloxacin, PRL = Piperacilin, SXT= Trimethoprim - sulphametaxzole(cotrimoxazole), C = Chloramphenicol, TZP = Piperacilin –tazobactam, TOB = Tobramycin , AUG = Augmentin(Amoxicilin- clavulnic acid), NOR = Norfloxacin , GEN = Gentamycin, MRP = Meropenem, IMP, Imipenem

5.7.1 MDR pattern of Non ESBL gram negative bacteria from mobile phones of HCWs

In the present study, a number of non ESBL producing gram negative bacteria isolates were obtained to have multidrug resistance characteristics 31.4%(87/277). 22.4% of the total isolates had shown resistance for only two antibiotics tested. In this study; 14.1% of them were found to be resistance for three drugs from different classes of antibiotics. Only 6% of *E.coli* was resistant for more than 5/13 antibiotics tested on the other hand 22.6% for one, 19% for two and 10.7% for three antibiotics had shown resistance. There were no bacterial isolate which show 100% MDR characteristic except *P. retgeri* which was resistant for four

antibiotics tested. Many of *Shigella spp* (37%) of them had MDR characteristic and only one species were resistant for more than seven antibiotic tested (Table 6).

Table 6: Multidrug resistance pattern of gram negative bacteria isolated from mobile phones of HCWs

Bacterial isolate n=277	**Antibiotic Resistance pattern**							
	R0	R1	R2	R3	R4	R5	R6	>R7
E. coli n=84	28(33.	19(22.6	16(19)	7(8.3)	9(10.7	2(2.4	1(1.2)	2(2.4
K. Pneumoniae n=14	2(14.3	2(14.3	4(28.6	0	2(14.3	0	3(21.4	1(7.1
K. ozenae n=7	2(28.6	3(42.9	1(14.3)	1(14.3	0	0	0	0
K. oxytoca n=4	1(25	0	0	3(75	0	0	0	0
Shigella Spp n=43	17(39.5	5(11.6	5(11.6	8(18.6	6(14	1(2.3)	0	1(2.3
S. dysentriae n=4	0	1(25	2(50	0	1(25	0	0	0
Salmonella Spp n=14	7(50	1(7.1	3(21.4	3(21.4	0	0	0	0
Enterobacter Spp n=5	0	1(20	2(40	1(20	1(20	0	0	0
E. aerogens n=7	2(28.6)	0	1(14.3)	2(28.6)	1(14.3)	0	1(14.3	0
Citrobacter Spp n=5	0	2(40	1(20	1(20	1(20	0	0	0
C. diversus n=5	2(40	1(20	0	1(20	1(20	0	0	0
Pseudomonas Spp n=25	7(28	5(20	3(12	6(24	2(8	1(4)	1(4	0
Proteus vulgaris n=2	0	1(50	0	0	0	1(50	0	0
P. mirabilis n=4	0	1(25	1(25	1(25	1(25	0	0	0
Acinetobacter Spp n=18	6(33.3	6(33.3	1(5.6	2(11.1	3(16.7	0	0	0
S. marscens n=12	2(16.7	6(50	2(16.7	1(8.3	1(8.3	0	0	0
P. stuarti n=5	1(20	3(60	1(20	0	0	0	0	0
P. retgeri n=1	0	0	0	0	1(100	0	0	0
M.morganii n=11	1(9.1	3(27.3	3(27.3	2(18.2	1(9.1	1(9.1	0	0
Alcaligens Spp n=3	1(33.3	2(66.7	0	0	0	0	0	0
E.agglomerans n=4	2(50)	0	1(25	0	1(25	0	0	0
Total	81(29.2)	62(22.4	47(17)	39(14.1	32(11.6	6(2.2)	6(2.2)	4(1.4)

Key: Ro = resistance to one antibiotic, R1 = resistance to two antibiotic, R3 = resistance to three antibiotic, …, >R7 = Resistance to more than seven antibiotics.

5.8 Associated factors for bacterial contamination on gowns of HCWs

Out of 572 gowns analyzed; 156(27.3%) were from physicians, 293(51.2%) from nurses, 36 (6.3%) from medical laboratory personnel, 28(4.9%) from pharmacy personnel and 59(10.3%) other HCWs. Of these total gowns; 345(60.3%) had long sleeve gown and 227(39.7%) HCWs were those who had short sleeve gown. Interestingly, 477/572 (83.4%) gowns were contaminated with at least one gram negative bacteria. Male participants had high rate of contamination than female participants. There were also a significant association among gender and microbial contamination of their gowns (Table 7).

Higher gram negative bacterial contamination was shown among the age group between 31-40 years old HCWs followed by 41-50 years old and this was significantly associated among the age group between 31-40 year (p<0.021,CI, 1.124-4.282].

Out of 572 samples evaluated, a gram negative bacterial growth was observed in (83.4%) of gowns and 79.6% had a single type of colony, 20% had two types of colony while 0.4% had ≥3 colony types. There was no significant difference between contamination rates and gown types ($p \geq 0.05$) (Table 7).

Gram negative bacteria distribution from gowns among the different profession showed a higher dominance from nurses 253/293(86.7%), followed by gowns tested from the medical laboratory personnel's 30/36(83.3%) was statistically non-significant among the different professionals (p-value >0.05) (Table 7).

Out of 345 long sleeve gowns 285/345 (82.6 %) were contaminated with at least one gram negative bacteria, while and N?? (84.6%) of gowns with short sleeves were contaminated with more than one gram negative bacteria. There were no significant differences in microbial contamination according to the type of sleeves of gowns (P.≥0.479, CI 0.539-1.337) as shown on Table 7.

HCWs who serve 5-7 years showed a greater degree of contamination 97/112 (86.6 %) followed by those who served for 3-4 years 127/150 (84.7%) in the hospital, but there was no significant association between the service years of HCWs and whether or not their gowns were contaminated with at least one gram negative bacteria ($p>0.05$) (Table 7).

Even though, there was no statistically significant association between frequency of gown changing and bacterial contamination in this study; higher contamination rate was found among those who change their gowns every two weeks 122/147(84%) followed by weekly 239/286 (83.6%) and low contamination rate was found on these who change their gown every day 11/14(78.6%) (Table7).

Table 7: Associated factors for microbial contamination for positive cultures from Gown of HCWs

Variables	Contaminated n= 477	Not contaminated n=95	Bivariate analysis		P-value
			COR	95%CI	
Gender					
Male n=238(41.6)	208(87.4%)	30(12.6%)	1.675	1.048-2.678	0.031
Female n=334(58.4)	269(80.5%)	65(19.5%)	R		
Age(year)					
20-30 n=412	335(81.3%)	77(18.7%)	R		
31-40 n=116	105(90.5%)	11(9.5%)	2.194	1.124_4.282	.021
41-50 n=27	23(85%)	4(15%)	1.322	.444_3.932	.616
>51 n=17	14(82.4%)	3(17.6%)	1.073	.301_3.825	.914
Profession					
Medical doctor n=156	122(78.2%)	34 (21.8%	1.020	.205_3.095	.972
Nurse n=293	254(86.7%	39(13.3%	.612	.622_1.826	.379
M. Laboratory n=36	30(83.3%)	6(16.7%)	1.329	.336_2.839	.463
Pharmacy n=28	22(78.6%)	6(21.4%)	R		
Other n=59	49(83.1%)	10(16.9%)	0.732	.336_1.596	.433
Service Year					
1-2 n=190	152(80%)	38(20%)	R		
3-4 n=150	127(84.7%)	23(15.3%)	1.380	.782-2.438	.267
5-7 n=112	97(86.6%)	15(13.4%)	1.617	.844_3.096	.147
8-10 n=45	37(82.2%)	8(7.8%)	1.156	.498_2.686	.736
>10 n=75	64(85.3%)	11(14.7%)	1.455	.700_3.024	.316
Gown Type					
Short Sleeve n=227	192(84.6%)	35(15.4%)	R		
Long Sleeve n=345	285(82.6%)	60(17.4%)	0.849	0.539-1.337	0.479

Gown changing frequency					
Daily n=14	11(78.6%	3(21.4%)			
Every other day n=147	122(83%)	25(17%)	1.331	0.346-5.119	677
Weekly n=286	239(83.6%0	47(16.4%)	1.387	0.373-5.162	.626
every two weeks n=125	105(84%)	20(16%)	1.432	0.366-5.597	.606

Most gowns had been contaminated with a single type of negative bacteria but 95 /477 of them were contaminated with two types of gram negative bacteria. Maximum double isolate was found from short sleeve gowns 39/192 (20.3%). There were also two samples which have three isolates at a time of study and these two of them were from long sleeve gowns. Generally equal amount (20.3%) of multiple isolates per specimen was found from gowns with long sleeves and short sleeves (Fig 5).

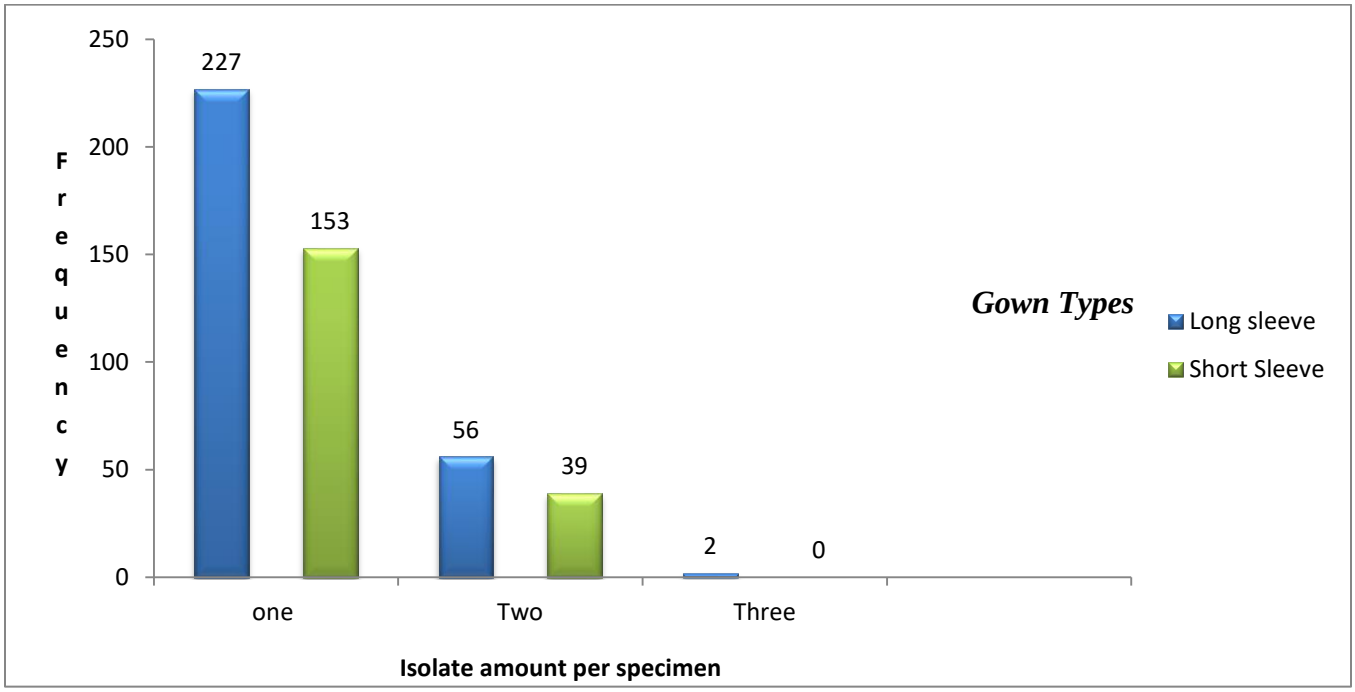

Figure 5: Distribution of bacterial isolates per specimen among Short sleeve and long sleeve gown

5.9 Distribution of ESBL producing gram negative bacteria from gowns of HCWs

Out of the total 572 gowns screened for ESBL producing gram negative bacteria, 57/572 (10%) was positive for ESBL using Screening test method for ESBL production. Among these; 54/572(9.44%)

isolates were confirmed for ESBL production using confirmatory test method (combination double disk method).

Out of the 54 confirmed ESBL producing gram negative bacteria; *K.Pneumoniae* was the dominant isolate 29.6% (16/54) followed by *E.coli* 9/54(16.7%) and *Citrobacter Spp* 8/54(14.8%), *Pseudomonas Spp* (14.8%), *K. oxytoca* (5.6%) and others were also identified in this study (Fig 6)

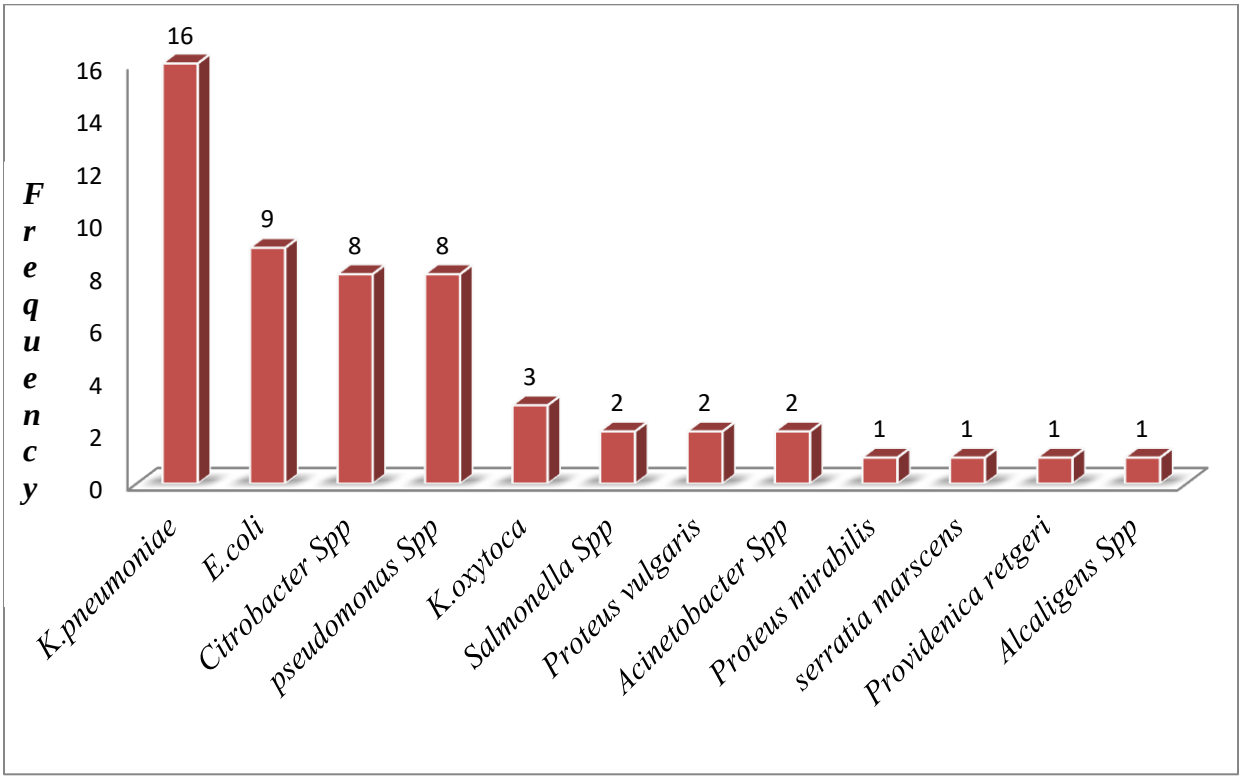

Figure 6: Distribution of ESBL producing gram negative bacteria isolated from gowns of HCWs

5.10 Antimicrobial susceptibility pattern of ESBL producing gram negative bacteria from gowns of HCWs

The resistance patterns of ESBL producing isolates from gowns of TASH HCWs against 13 antimicrobial agents are presented in Table 8. The highest degree of resistance in *K .pneumoniae* was seen against ampicillin 81% followed by cotrimoxazole (75%) and gentamycin (58%). All isolates except 1/8(12%) of *Pseudomonas Spp* were 100% resistant for ampicillin. *Salmonella Spp* was found to be 100% resistance to ampicillin, cefoxitin, co-trimoxazole, Chloramphenicol and amoxicillin-clavulnic acid on the other hand it was 100% sensitive for amikacin, ciprofloxacin, piperacilin, tobramycin, norfloxacin, gentamycin and imipenem. For *Citrobacter spp*, even though there were 100% sensitive

for piperacilin –tazobactam ,(88%), for amikacin, ciprofloxacin and tobramycin each; higher resistance were also found for ampicillin(100%), chloramphenicol (75%) and cotrimoxazole (62%).one species of *P. retgeri* was found alarmingly highly resistant for all antibiotics tested except amikacin and piperacilin –tazobactam (Table 8).

Table 8: AST pattern of ESBL producing gram negative bacteria from Gowns of HCWs

Species	**AST**	**Antibacterial agents**													
		AMK	AMP	FOX	CIP	PRL	SXT	C	PZT	TOB	AUG	NOR	GEN	MRP	IMP
E.coli n=9	S	6(67)	0	3(33)	4(44)	4(44)	6(67)	6(67)	8(89)	7(78)	6(67)	5(56)	6(67)	8(89)	8(89)
	I	1(11)	0	0	0	1(11)	0	0	0	1(11)	0	0	0	0	0
	R	2(22)	9(100	6(67)	5(56)	4(44)	3(33)	3(33)	1(11)	1(11)	3(330	4(44)	3(33)	1(11)	1(11)
K. pneumoniae n=16	S	14(87	2(13)	10(62	10(62	6(38)	3(19)	5(31	15(94)	10(62	8(50)	13(81	6(36	10(62	11(68
	I	0	1(6)	0	1)6	1(6)	1(6)	3(19)	1(6)	0	1(6)	1(6)	1(6)	2(13)	2(13)
	R	2(13)	13(81	6(38)	5(32)	9(56)	12(75	8(50)	0	6(38)	7(44)	2(13)	9(58)	4(25)	3(19)
K. oxytoca n=3	S	3(100	0	2(68	3(100	3(100	0	0	3(100	3(100	3(100	3(100	2(68	3(100	2(68
	I	0	0	0	0	0	0	0	0	0	0	0	1(32	0	0
	R	0	3(100	1(32	0	0	3(100	3(100	0	0	0	0	0	0	1(32
Salmonella Spp n= 2	S	2(100	0	0	2(100	2(100	0	0	0	2(100	0	2(100	2(100	0	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	2(100	2(100	0	0	2(100	2(100	2(100	0	2(100	0	0	2(100	0
Citrobacter SPP n=8	S	7(88)	0	6(75)	7(88)	6(75)	3(38)	2(25)	8(100	7(88)	5(62)	5(62)	6(75	7(77	6(75
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(12)	8(100	2(25)	1(12)	2(25)	5(62)	6(75)	0	1(12)	3(38)	3(38)	2(25)	1(13)	2(25)

Pseudomonas Spp n=8	S	8(100	1(12	5(62)	8(100	4(50	2(25)	4(50	8(100	4(50	5(62)	4(50	5(62)	6(75)	5(62)
	I	0	0	0	0	0	0	1(12)	0	0	0	0	0	0	0
	R	0	7(88)	3(38)	0	4(50	6(75)	3(38)	0	4(50	3(38)	4(50	3(38)	2(25	3(38)
P.vulgaris n=2	S	2(100	0	0	2(100	0	0	1(50	2(100	1(50	1(50	0	2(100	1(50	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	2(100	2(100	0	2(100	2(100	1(50	0	1(50	1(50	2(100	0	1(50	2(100
P.mirabilis n=1	S	1(100	0	1(100	0	0	0	0	1(100	1(100	0	1(100	1(100	1(100	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	0	1(100	1(100	1(100	1(100	0	0	1(100	0	0	0	1(100
Acinetobacter Spp n=2	S	1(50	0	1(50	1(50	1(50	0	1(50	1(50	2(100	1(50	0	1(50	0	2(100
	I	0	0	0	1(50	0	0	0	0	0	0	1(50	0	0	0
	R	1(50	2(100	1(50	0	1(50	2(100	1(50	1(50	0	1(50	1(50	1(50	2(100	0
S.marscens n=1	S	1(100	0	1(100	0	1(100	1(100	0	1(100	1(100	0	1(100	1(100	0	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	0	1(100	0	0	1(100	0	0	1(100	0	0	1(100	0
P.retgeri n=1	S	1(100	0	0	0	0	0	0	1(100	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	1(100	1(100	1(100	1(100	1(100	0	1(100	1(100	1(100	1(100	1(100	1(100
Alcaligens n=1	S	1(100	0	0	0	0	0	0	1(100	1(100	1(100	1(100	1(100	1(100	1(100
	I	0	0	1(100	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(100	0	1	1(100	1(100	1(100	0	0	0	0	0	0	0

Key: S= sensitive, R= Resistance, I= Intermediate, AMP=Ampicillin, AMK= Amikacin, FOX = Cefoxitin, CIP= ciprofloxacin, PRL = Piperacilin, SXT= Trimethoprim - sulphametaxzole(cotrimoxazole), C = Chloramphenicol, TZP = Piperacilin –tazobactam, TOB = Tobramycin , AUG = Augmentin(Amoxicilin- clavulnic acid), NOR = Norfloxacin , GEN = Gentamycin, MRP = Meropenem, IMP, Imipene

5.10.1 MDR pattern of ESBL producing bacterial isolates from gowns of TASH HCWs

Multidrug resistance (resistant to three or more antibiotics from different classes of antibiotics) was observed among 90.7% (49/55) of gram negative bacterial isolates. Among the tested antibiotics the

highest degree of resistance was seen against, ampicillin, co-trimoxazole, chloramphenicol, piperacilin, cefoxitin and augmentin respectively. The overall range of susceptibility for different antibiotics was from 0% to 100%.Amongst all the bacterial isolates, *S. marscens* (100%), *Salmonella Spp*(100%), *Acinetobacter Spp* (100%) and *K. pneumoniae* (87.4%) showed MDR characteristics.

18.8% *K. pneumoniae*, 25% *Pseudomonas Spp*, 50% had a resistance for more than eight antibiotics tested in this study exhibited resistance against more than five drugs (Table 9).

Table 9: MDR pattern of ESBL producing gram negative bacteria isolated from gowns of HCWs

Bacterial isolates	**MDR Resistance pattern n(%)**							
	R1	R2	R3	R4	R5	R6	R7	>R8
E.coli n=9	1(11.1)	0	0	2(22.2)	1(11.1)	4(44.4)	1(11.1)	0
K.Pneumoniae n=16	1(6.3	1(6.3	2(12.5)	3(18.8)	1(6.3)	3(18.8)	2(12.5)	3(18.8)
K. oxytoca n=3	1(33.3	0	1(33.3)	1(33.3)	0	0	0	0
Salmonella Spp n=2	0	0	0	0	2(100)	0	0	0
Citrobacter Spp n=8	0	1(12.5	2(25)	2(25	1(12.5)	1(12.5)	1(12.5)	0
pseudomonas Spp n=8	0	0	3(37.5)	1(12.5)	1(12.5)	1(12.5)	0	2(25)
P. vulgaris n=2	0	0	0	0	0	1(50	0	1(50
P. mirabilis n=1	0	0	0	0	0	0	1(100	0
Acinetobacter Spp n=2	0	0	0	1(50)	0	0	0	1(50)
S. marscens n=1	0	0	0	0	1(100)	0	0	0
P. retgeri n=1	0	0	0	0	0	0	0	1(100)
Alcaligens Spp n=1	0	0	0	0	0	1(100)	0	0
Total	3(5.6)	2(3.7)	8(14.8)	10(18.5)	7(13)	11(20.4)	5(9.3)	8(14.8)

Key: Ro = resistance to one antibiotic, R1 = resistance to two antibiotic, R3 = resistance to three antibiotic, …, >R8 = Resistance to more than Eight antibiotics.

5.11: Gram negative bacteria profile from gowns of HCWs

Beside to identification of ESBL from a total 576 bacterial isolates from Gowns of TASH HCWs; 280non ESBL producing bacterial isolates were selected for bacterial identification and Antibacterial susceptibility pattern using systematic random sampling. Out of these 280 bacterial isolates the most predominant gram negative bacteria was *E.coli* 62/280(22%) followed by *Acinetobacter Spp* 59/280(21%) and *S.dysentriae* 33/280(11.7%) respectively. Many gram-negative bacilli which cause hospital acquired infections such as *Klebsiella Spp, Enterobacter Spp, Citrobacter Spp, E.aerogens, , Pseudomonas Spp, Salmonella Spp, Proteus Spp, P. stuarti, S. marscens and Alcaligens* were identified in this study (Fig:7).

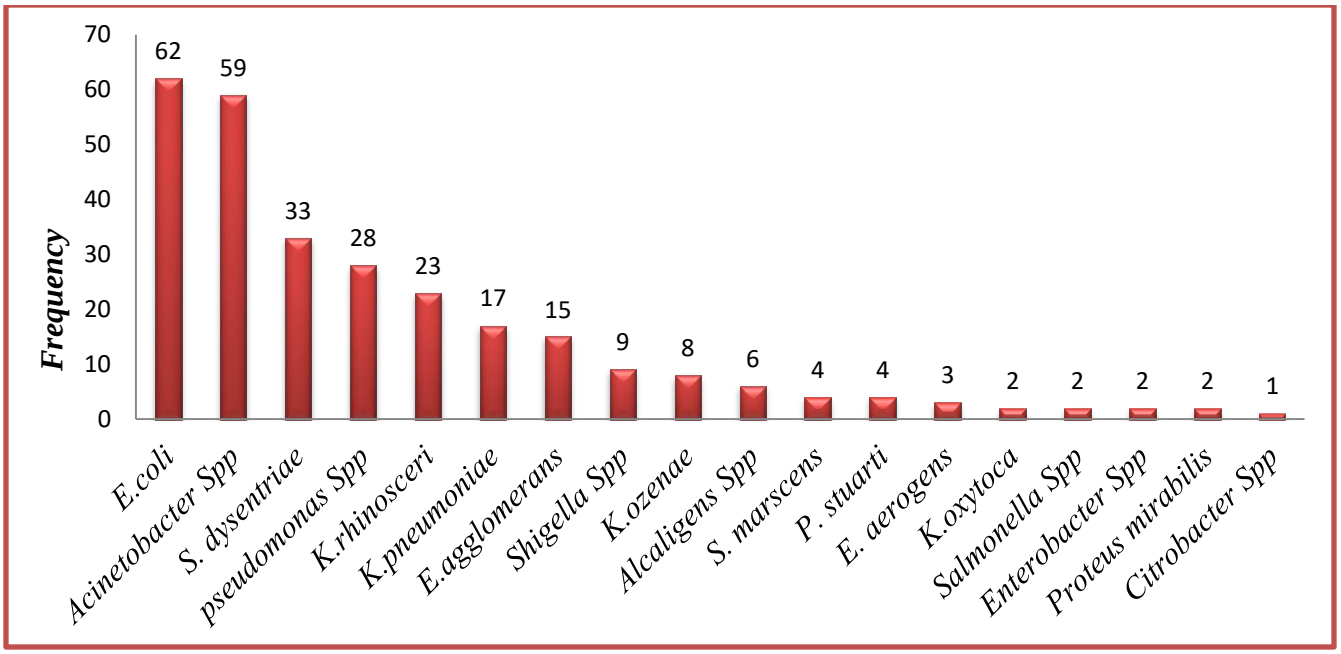

Figure 7: Distribution of gram negative bacteria from gowns of TASH HCWs

5.12 Antimicrobial susceptibility pattern of gram negative bacterial isolates from Gowns of HCWs

In this current study, susceptibility pattern of gram negative isolates towards 280 isolates were carried out from gowns of HCWs as recommended by CLSI 2018. The predominant gram negative bacteria isolate were *E.coli* showed that a sensitivity ranges from 76%-98% of all tested antibiotics. From the 59 isolated *Acinetobacter Spp* only one species were resistant for meropenem and imipenem each; the rest of the species showed a resistance which ranges from 31%-56%. Alarmingly a very high level of resistance was shown in *E.aerogens*,100% resistance For AMP, FOX, CIP, PRL, TOB, AUG, NOR and GEN (Table 10).

The carbapenems were the most active antibiotic against *E.coli, K. pneumoniae, pseudomonas Spp* and other identified bacteria with a sensitivity range 90% and 100% of all gown isolates. Piperacilin-tazobactam was also an active antibiotic of the penicillin-derived compounds, with total susceptibility ranging from 90% to 100% (Table 10).

Table 10: AST pattern of gram negative bacteria isolates from gowns of HCWS

Species	AST	Antibacterial agents													
		AMK	AMP	FOX	CIP	PRL	SXT	C	PZT	TOB	AUG	NOR	GEN	MRP	IMP
E.coli n=62	S	58(94	47(76	51(82	51(82	50(81	51(82	58(94	61(98	49(79	48(77	50(81	54(87	61(98	61(98
	I	2(3	0	2(3)	0	0	1(2	0	0	0	0	0	0	0	0
	R	2(3	15(24	9(15)	11(18	12(19	10(18	4(6	1(2	13(21	14(23	12(19	8(13)	1(2)	1(2)
K. pneumoniae n=17	S	14(82	13(76	14(82	11(64	10(59	11(64	13(76	17(100	15(88	13(76	15(88	11(64	17(100	17(100
	I	1(6	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	2(12	4(24	3(18	6(36	7(31	6(26	4(24	0	2(12	4(24	2(12	6(26	0	0
K.ozenae n=8	S	8(100	4(50	6(75	4(50	4(50	4(50	6(75	8(100	7(88	8(100	8(100	7(88	8(100	8(100
	I	0	0	0	0	0	0(50	0	0	1(12	0	0	0	0	0
	R	0	4(50	2(25	4(50	4(50	4	2(25	0	0	0	0	1(12	0	0
K.oxytoca n=2	S	2(100	2(100	1(50	1(50	1(50	1(50	2(100	2(100	1(50	0	1(50	1(50	2(100	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	1(50	1(50	1(50	1(50	0	0	1(50	2(100	1(50	1(50	0	0
K.rhinoceri n=23	S	22(96	13(57	23(100	18(78	15(65	18(78	19(83	23(100	16(70	18(78	19(83	17(74	23(100	23(10
	I	0	0	0	0	0	0	0	0	0	0	1(4)	0	0	0
	R	1(4	10(43	0	5(22	8(35	5(22	4(17)	0	7(30)	5(22	3(13)	6(26)	0	0
Shigella Spp n=9	S	8(89	6(67	6(67	8(89	6(67	6(67	9(100	8(89	8(89	7(78	9(100	8(89	9(100	9(100
	I	0	0	0	0	1(11)	0	0	1(11)	0	1(11)	0	0	0	0
	R	1(11	3(33)	3(33)	1(19	2(22)	3(33)	0	0	1(11)	1(11)	0	1(11	0	0
S. dysentriae n=33	S	27(82	21(64	21(64	23(70	15(45	24(73	23(70	33(100	24(73	22(67	24(73	22(67	33(100	33(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	6(18	12(36	12(36	10(30	18(55	9(27	10(30	0	9(27	11(33	9(27	11(33	0	0

Salmonella Spp n=2	S	2(100	1(50	1(50	1(50	1(50	2(100	1(50	2(50	1(50	1(50	1(50	1(50	2(100	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	1(50	1(50	1(50	1(50	0	1(50	0	1(50	1(50	1(50	1(50	0	0
Enterobacter Spp n=2	S	2(100	2(100	2(100	1(50	2(100	1(50	2(100	2(100	2(100	1(50	1(50	2(100	2(100	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	1(50	0	1(50	0	0	0	1(50	1(50	0	0	0
E .aerogens n=3	S	3(100	0	0	0	0	3(100	3(100	3(100	0	0	0	0	3(100	3(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	3(100	3(100	3(100	3(100	0	0	0	3(100	3(100	3(100	3(100	0	0
Citrobacter Spp n=1	S	1(100	1(100	1(100	0	1(100	0	1(100	1(100	0	1(100	1(100	1(100	1(100	1(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	1(100	0	1(100	0	0	1(100	0	0	0	0	0
Pseudomonas Spp n=28	S	24(86	14(50	18(64	18(64	18(64	18(64	20(71	28(100	22(79	24(86	22(79	23(82	28(100	28(10
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	4(14	14(50	10(36	10(36	10(36	10(36	8(29	0	6	4(14	6(21	5(18	0	0
P. mirabilis n=2	S	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100	2(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acinetobacter Spp n=59	S	40(68	28(47	26(44	28(47	28(47	28(47	41(69	28(47	33(56	32(54	34(57	29(49	57(96	57(96
	I	0	1(2	0	1(2	0	0	0	0	0	0	0	1(2	1(2	1(2
	R	19	30(51	33(56	30(51	31(53	31(53	18(31	31(53	26(44	27(46	25(43	29(49	1(2	1(2
S.marscens n=4	S	3(75	3(75	3(75	3(75	4(100	3(75	3(75	4(100	4(100	3(75	3(75	3(75	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(25	1(25	1(25	1(25	0	1(25	1(25	0	0	1(25	1(25	1(25	0	0

P. stuarti n=4	S	3(75	2(50	1(25	2(50	2(50	2(50	2(50	4(100	2(50	1(25	2(50	2(50	4(100	4(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(25	2(50	3(75	2(50	2(50	2(50	2(50	0	2(50	3(75	2(50	2(50	0	0
Alcaligens Spp =6	S	5(83	4(67	4(67	4(67	6(100	4(67	4(67	6(100	5(80	5(83	3(50	5(83	6(100	6(100
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	R	1(17	2(33	2(33	2(33	0	2(33	2(33	0	1(17	1(17	3(50	1(17	0	0
E.agglomerans n=15	S	11(73	11(73	13(87	10(67	9(60	8(53	12(80	14(93	10(67	10(67	11(73	9(60	14(93	14(93
	I	0	0	0	0	0	0	1(7	0	0	0	0	0	0	0
	R	4(27	4(27	2(13	5(33	6(40	7(47	2(13	1(7	5(33	5(33	4(27	6(40	1(7	1(7

Key: S= sensitive, R= Resistance, I= Intermediate, AMP=Ampicillin, AMK= Amikacin, FOX = Cefoxitin, CIP= ciprofloxacin, PRL = Piperacilin, SXT= Trimethoprim - sulphametaxzole(cotrimoxazole), C = Chloramphenicol, TZP = Piperacilin –tazobactam, TOB = Tobramycin , AUG = Augmentin(Amoxicilin- clavulnic acid), NOR = Norfloxacin , GEN = Gentamycin, MRP = Meropenem, IMP, Imipenem

5.13 MDR resistance pattern of Non –ESBL gram negative bacteria from gowns of TASH HCWs

Around 49.3% (138/280) isolates were found to be resistant to three and more than 3 antibiotics from different class of antibiotics. About 13.9% of the total isolates were resistant to four different antibiotics and 4.6% of total isolates also showed a resistant pattern to more than 11/14 antibiotics tested. Eighty three percent (49/59) isolates of *Acinetobacter Spp* showed MDR characteristic for three or more than three tested antibiotics.100% *E. aerogens* showed a resistance to eight tested antibiotics.

P. stuarti, *E. agglomerans and S. dysentriae* respectively had shown a resistance rate of 25%, 13.3%, and 9.1% respectively to eleven and above tested antibiotics in this study (Table 11).

Table 11: MDR resistance pattern of gram negative bacteria from gowns of HCWs, 2019

Bacterial Isolates n=280	**Antibiotic resistance pattern**										
	R0	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R8**	**R9**	**R10**	**>R11**
E. coli n=84	26(42)	13(21)	10(16.1	3(4.8	4(6.5	0	1(1.6	2(3.2	1(1.6	1(1.6	1(1.6
K. Pneumoniae n=17	2(11.8	2(11.8	4(23.5)	4(23.5	2(11.8	2(11.8	1(5.9	0	0	0	0
K. ozenae n=8	0	2(25	2(25)	1(12.5	3(37.5	0	0	0	0	0	0
K. oxytoca n=2	0	1(50)	0	0	0	0	0	1(50	0	0	0
K. rhinosceri n=23	5(21.7	2(8.7	4(17.4	5(21.7	6(26.1	1(4.3	0	0	0	0	0
Shigella Spp n=9	4(44.4	1(11.1	1(11.1	1(11.1	0	2(22.2	0	0	0	0	0

S. dysentriae n=29	5(15.2	4(12.1	5(15.2	4(12.1	7(21.2	3(9.1	1(3	0	1(50	0	3(9.1
Salmonella Spp n=2	1(50	0	0	0	0	0	0	0	1(3	0	0
Enterobacter Spp n=2	0	0	2(100	0	0	0	0	0	0	0	0
E. aerogens n=3	0	0	0	0	0	0	0	3(100	0	0	0
Citrobacter Spp n=1	0	0	0	1(100	0	0	0	0	0	0	0
pseudomonas Spp n=28	3(10.7)	1(3.6	8(28.6	4(14.3	7(25	0	5(17.9	0	0	0	0
Proteus mirabilis n=2	2(100)	0	0	0	0	0	0	0	0	0	0
Acinetobacter Spp n=59	5(8.5)	5(8.5)	6(10.2)	11(19)	7(12)	2(3.4)	5(8.5)	3(5.1	4(6.8	5(8.5	6(10.2
S.marscens n=4	3(75)	0	0	0	0	0	0	0	1(25	0	0
P.stuarti n=4	1(25	0	0	0	1(25	0	0	1(25	0	0	1(25
AlcaligensSpp n=6	0	2(33.3	1(16.7	1(16.7	1(16.7	0	1(16.7	0	0	0	0
E.agglomerans n=15	3	3(20	1(6.7	3(20	1(6.7	1(6.7	1(6.7	0	0	0	2(13.3
Total	60(21.4	36(13)	44(15.7	38(13.6	39(14)	11(3.9	15(5.4	10(3.6	8(2.9	6(2)	13(4.6

Key: Ro = resistance to one antibiotic, R1 = resistance to two antibiotic, R3 = resistance to three antibiotic… >R11 = Resistance to more than Eleven antibiotics.

6.0 Discussion

Cell phones have become one of the essential devices used for communication in daily life, and they are commonly used almost everywhere. HCWs use these phones for communication within hospital settings and also many medical conditions like diabetes, asthma and an increased rate of vaccination by travelers reminded by short message service (SMS) have been controlled after the innovations of mobile communications [37, 38, 39]. However, one of the most common concerns regarding use of mobile devices in hospital setting and outside is; they can act as a vehicle for transmitting pathogenic microorganisms [14, 28, 31]. However, there are no institutional or manufacturer protocols that advise on strategies to reduce the surface bio burden on mobile devices, or indeed other clinician associated devices such as stethoscopes in our setting.

The percentage of gram negative bacteria contamination to at least one bacterium on the tested cell phones was 454/572(79.4%). Even though, there were no studies specifically done for gram negative bacteria, this frequency is relatively high to those previously reported in Ethiopia and other countries [24, 25]. We found that 82.3% mobile phones of females were more contaminated than mobile phones (75.2%) of men, which is statistically significant[P-value=0.039, CI 0.43 – 0.978]. Study on female HCWs showed that their handbags could play a role in bacterial transmission and that mobile phones can be further contaminated by other materials carried inside handbags [40, 41, 42]. A significantly associated result (P =0.044, CI 1.044-18.542) between microbial contamination rate among Medical laboratory personnel 91.7% (33/36) followed by nurses across the different professions was found (Table 2) and this was supported by another study done in Egypt [32].

Even though, it was not significantly associated (P>0.05) there was higher contamination rate of mobile phones with at least one gram negative bacteria among HCWs who serve for more than 10 years 64/75(85%) compared to those who serve less than five years. Interestingly we found a higher contamination of mobile phones among age group of >50 years old (Table 2). Our study differs from a previous study conducted in Harar, Eastern Ethiopia which revealed that the age group between 25-49 years had more contamination [42].

Out of 107 KMP 89 (83.2%) of them were found more contaminated compared to TMP (78.5%) 365/465 (Table 2). Different studies had focused on the effects of MP design in terms of touch screen versus key pad mobile phones with respect to bacterial contamination (44, 45, 46). Some studies showed that smart phones were more contaminated by microorganisms than non-smart phones, and suggested

that this may be caused by the wider screen and more intense usage pattern of the former even though our study didn't show significant association (P-value >0.05) it disagrees with other studies conducted on effect of mobile size and contamination rate[44,45]. But also in line to a study conducted by Pal *et al*; found that TMP had lower bacterial contamination when compared with KMP, which they reported as a reason for this high contamination in KMP than IMP was due to the more complex surface structure of the KMP [46].

ESBLs have become widespread and cause serious infection. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromises the activity of wide-spectrum antibiotics creating major therapeutic difficulties with significant challenge. Many different ESBL producing gram negative bacteria were identified in this study. The MDR bacteria identified on these mobile phones suggest that phone contamination is a marker of the nosocomial pathogens that circulate in the hospital environment and many different studies support this finding locally as well as internationally [29-32, 47].

In the current study 79.4% (454/572) mobile phones were contaminated to at least one gram negative bacteria. Among these 8.3 %(48/572) were ESBL producing bacterial species. This frequency was low compared to similar study conducted in Peru (33.3%) [29]. In this current study the most dominant isolate was *K.Pneumoniae* (28%) followed by *E.coli* (14.6%) and *Acinetobacter spp* (14.6%) (Fig: 2). Even though this frequency is high; different studies had reported the presence of ESBL producing gram negative bacteria in their studies [28, 30, 31, 32]. High prevalence of ESBL producing gram negative bacteria from mobile phones was observed comparing to previous studies conducted in Ethiopia (25, 42). The difference between our study and studies conducted in our country could be the study area, the patient handling rate; since earlier studies were conducted in health centers and the sample size they used were also smaller than ours. In our study, 79.2% of ESBL-producing isolates showed multidrug resistance to at least one drug from different families of antibiotics (>3 antibiotics from different classes) (Table 4). This was similar with a study conducted by Asmari *et al*; they revealed 71.8% of the isolates were MDR bacteria [30]. Similar to studies by Selim *et al*; and Gashaw *et al*; high degree of resistance was shown for ampiciilin, cotrimoxazole and chloramphenicol [24, 32]. In the present study most of the ESBL isolates were sensitive to imipenem and meropenem. Among β-Lactam inhibitor drugs highest sensitivity (100%) was observed with piperacilin-tzobactam than amoxycillin- clavlnic acid in case of all isolates except *P.vulgaris* (50%) and *Citrobacter Spp* (80%) while high resistance (100%) was

observed with amoxicillin-clavulnic acid for *S. dysentriae, Sallmonella Spp, P. mirabilis and Acinetobacter Spp* (Table 3). These findings were also in agreement with other studies [48-50]. But bacterial species like *Acinetobacter*, *Pseudomonas* and *E.coli* had shown slight resistance to carbapenem and β-lactam inhibitors. This is could be because some bacteria have an ability to create a resistance mechanism for different antibiotic classes. These are diminished production of outer membrane porin, possession of efflux pump, production of carbapenemase and β-lactamase [51, 52]. Colonization of such devices by potential pathogens like ESBL producing gram negative bacteria indicates the possible spread of these pathogens among the hospital as well as in community setting (47).

In addition to identification of ESBL producing gram negative bacteria, we also looked at the distribution and AST pattern of Non ESBL producers from mobile phones. In our study, *E. coli* (30.3%), *Shigella Spp* (15.5%), *Pseudomonas Spp* (9.1%), *K. pneumonia (*5.0%) and *Acinetobacter spp* (5.0%) were isolated (Fig: 4). Other studies performed in hospitals in other countries also have isolated these bacteria from mobile phones of HCWs. In Ethiopia by Gashaw *et al; E. coli* (6.8%), *P. stuartii* (5%), *K. pneumoniae* (3.4%), *Citrobacter* (3.4%) *and E. cloacae* (3.4%). Another study in Ethiopia by Bodena *et al*; *K. pneumoniae* (6.9%), *E. coli* (5.6%), *Pseudomonas Spp*(3.5%) and *Citrobacter Spp*(3.5%).In Nigeria by Nwaknko *et al*; *Pseudomonas Spp*(19.6%), *K. pneumoniae* (7.1%), E. coli (14.3%), *Acinetobacter Spp* (5.3%) and *Proteus Spp* (12.5%). In India, *P. aeruginosa (*15%), *E. coli (*11 %) and *K. pneumoniae (*10%). In Turkey, Karabay *et al;* isolated *E. coli*, *P. aeruginosa* and *K. pneumoniae* in 3.6%; 1.8% and 0.9% respectively (24, 43, 53 ,56). The finding in the current study is a very higher incidence compared to different studies conducted (19, 54 , 55). This higher incidence comparing to these studies could be due to lack of awareness, absence of policies and regulation that restrict using mobile phones in our setting.

K. pneumoniae, Proteus spp, Citrobacter Spp, Enterobacter Spp, E. agglomerans and Providencia, which are commonly found in hospital-acquired infections, were observed in this study(Fig: 4) and were also reported by other researchers [53, 42, and 57]. Bacterial contamination in phones of HCW's was frequent and diverse, leading to a large number of isolates. This could suggest that phones may be sensitive indicators to monitor bacterial contamination in settings where phones are frequently used, not regularly disinfected, and exposed to the environment.

Even though, there is no supporting previous study conducted from mobile phones of HCWs; we found a number of gram negative bacteria like *Alcaligens Spp* 1/277(1.1%), *M. morganni* 11/277(4%), *K. oxytoca* 4/277 (1.4%), *K. ozenae* 7/277(2.5%), *Shigella Spp* (15.5%), *S. marcscens* 12/277(4.3%) and *S. dysentriae* 4/277(1.4%) from mobile (Fig:4). Environmental surfaces in healthcare facilities act as a reservoir for bacteria and it serve as vectors for pathogenic microorganism. Since the hospital environment can be contaminated with pathogenic bacteria mentioned above, HCWs mobile phones, gowns and other inanimate objects could be contaminated. Micro-organisms can be transferred from person to person or from inanimate objects (such as bronchoscopes, pagers, ballpoint pens, stethoscopes, patient hospital charts ,computer keyboards, mobile phones) to hands and vice versa [48,58].

As per the reports of study conducted on bacterial contamination of mobile phones used by HCWs, of the tested antimicrobials, ampicillin, chloramphenicol followed by cotrimoxazole were resistant by many of the isolates of gram negative bacteria [24, 25]. This is in line with the finding of the current study since most of our isolates had shown resistance for ampicillin, chloramphenicol and cotrimoxazole respectively (Table 5). Multidrug resistance characteristic was observed among many species of gram negative bacteria to multiple classes of drugs. But alarmingly very high rate of MDR was shown among *K. oxytoca* (75%), *E. aerogenes* (57.2%). Most of the isolates were sensitive for carbapenems and a β-lacatam inhibitors; piperacillin -tazobactam (92%-100%) tested in this study. Many studies also support the present finding (24, 30, 32). Multidrug resistance observed by the remaining species of gram-negative bacteria as presented in Table 6 was 21(25%) for *E.coli* (27.8%) *Acinetobacter spp* and 10(40%) for *Pseudomonas spp* which was considered significant. Generally, multidrug resistance among gram-negatives was 31.4%.

The findings of this hospital based study indicate that gowns used by HCWs can serve as a reservoir of a very high load of bacterial agents and could play an important role in the transmission of HAI in healthcare settings. This current study elaborates on earlier research suggests that HCWs gowns could contribute to transmitting gram negative pathogenic bacteria in hospital settings [21, 33-35, 59-65].

In our study, up to 83.4% of the gowns screened were contaminated with at least one gram negative bacteria. Even though, there was no published study in our country but different studies from other countries had reported gram negative bacteria from gowns of HCWs ranging from 0.5- 50% [21, 33-35, 59, 60, 61]. The rate of bacterial contamination of HCWs white coats may be associated with patients'

continuously shedding off infectious microorganisms in the hospital environment, ability of microorganisms to survive 10-98 days on surface and fabric materials such as cotton or polyester. Some researchers have suggested that a patient skin can be a source of contamination for the HCWs white coat during contact with patients [15, 21, 61, 62].

The gowns of the male (87.4%) HCWs were more contaminated than females (80.5%) participants (P-value =0.031, CI, 1.048-2.678) as shown in table 7. Though similar finding has been reported [15, 35, 62, 63] but some studies are contradictory to the current findings [64, 65]. The fact that male HCWs are of the habit of keeping some of their belongings such as pens, mobile phones, stethoscopes and other portable devices in their pockets but female on the other hand have alternative way of keeping all these items on their hand bags.

The rates of gram negative bacteria contamination of those that change their white coats every two week had a higher contamination than those who change every week and the like every day and every other day (Table 7). Other reports are also consistent with our study [21,35]. It had shown slightly inconsistent with previous studies [33, 66, 67]. Although, there were no significant association between frequencies of HCWs change their gowns and whether or not gowns would be contaminated, individual disposition to their white coats handling can be adduced as reason independent of the length of use [69]. A possible reason for this could be that it is not the number of white coats, but how frequently a particular white coat is washed that relates to contamination. This idea was also supported by Noor F *et al* [65].

There were no significant association between bacterial contamination of gowns and service year of HCWs on this current study but higher contamination of bacteria was found among those who serve 5-7 years(86.6%) followed by >10 Years (85.3%) in the hospital as shown in table 7. A similar study in line with current study revealed as there is no association among year of experience, education level, and experience level [68].

Nurses (86.7%) gowns were found to be the highest contaminated as compared to the other personnel of HCWs followed by Medical laboratory personnel. This agrees with the findings of some authors [21, 60, 63]. The fact that Nurses have closest contact with patients compared to other HCWs might explain the high contamination occurrence among them. Laboratory scientists had also a higher rate of contamination next to nurses and this is expected to have contact with the patient's body fluids as they

are assigned with the responsibility of examining of samples. This could suggest that there is a relationship between gowns and handling practices and bacterial contamination.

In our study we found that that long-sleeved white coats were heavily less contaminated than were short-sleeved uniforms but this was found statistically non-significant [P-value =0.479,CI 0.539-1.337]. Our study disagrees with a study conducted by Munoz-Price LS *et al*, [59]. Whereas it is in agreement with another study conducted on white coats of HCWs [69].

Gowns of health care workers have been shown to carry and serve as a vehicle for potential gram negative bacteria contamination and a role in the nosocomial transmission of pathogenic multi-drug resistant bacteria like ESBL, MRSA and VRE (33). It is well accepted that proper handling of white coats by physicians and other HCWs could minimize cross- contamination and improve patient safety by potentially reducing nosocomial infections.

On this current study 9.4% ESBL producing species of gram negative bacteria were isolated. *K. pneumoniae* was the dominant isolate 29.6 % followed by *E.coli* (16.7%) and *Citrobacter spp* (14.8%), *Pseudomonas spp* (14.8%), *K.oxytoca* (5.6%) as described in Fig: 6. Our finding was lower compared to study conducted in Tanzania by Silago *et al ;*(22.5%) where the dominant bacteria was *Citrobacter freundi* followed by *Acinetobacter Spp* [70] which disagrees with our finding. On the other hand our finding is higher compared to studies conducted in Nigeria, Tanzania and Egypt [21,34 , 35]. Similar studies have also shown the presence of *K. pneumoniae*, *Acinetobacter spp*, *Enterobacter spp*, *Pseudomonas spp* and *E coli* on other inanimate objects and hospital environments [71,72].

The reason for the high occurrence of ESBL producing bacteria could be; HCWs contaminated hands with patients, clinical coats contaminated with hospital surface materials, mobile phones and medical examination equipment's in routine uses since these materials could become contaminated with ESBL producing gram negative bacteria when they come into contact with infected or colonized patients' surfaces and subsequently act as reservoir or vehicle of transmission to patients [15,73-75].

Multidrug resistance was observed among 90.7% (49/55) of gram negative bacterial isolates. Among the tested antibiotics the highest degree of resistance was seen against, ampicillin, cotrimoxazole, chloramphenicol (Table 8).

In the current study, ESBL producing gram negative bacteria were highly sensitive to meropenem, imipenem and piperacilin- tazobactam and moderately sensitive to amikacin and less sensitive to ampicillin, cotrimoxazole and chloramphenicol (Table8). Similarly, lower sensitivity of ESBL producing gram negative bacteria to ampicillin were previously reported [70, 76-77]. Being cheap and first line of treatment, make these antibiotics to be highly misused and therefore bacteria could be more likely to develop resistance [78]. As observed previously, ESBL producers are highly sensitive to carbapeneme and β-lactam inhibitors [79,80]. Infrequent availability and expensive cost of meropenem and imipenem in most of healthcare settings and pharmacies in developing countries, limit its misuse and extreme use, therefore, there will be limited or no resistant against meropenem [80]. However, in our study some ESBL producing gram negative bacteria like *E.coli, K. oxytoca, K. pneumoniae, Acinetobacter Spp and pseudomonas spp* had shown resistance to carbapenem. This could be due to the ability of some bacteria like *Acinetobacter spp* to simultaneously equip itself with intrinsic and extrinsic mechanisms of resistance to antibacterial agents. This includes diminished production of outer membrane porin, possession of efflux pump, production of carbapenemase and beta lactamase [51, 52]. Contaminated clinical coats can act as vehicles to carry and transmit ESBL producing gram negative bacteria from one patient to another, to oneself (medical personnel) and to the community to become the ultimate source of community acquired multidrug resistant bacteria as reported before [70,72 ,75].

Beside to identification of ESBL we also tried to study the distribution and AST pattern of non ESBL producers from mobile phones. We detected many different pathogens, which are commonly related to nosocomial infection (e.g. *E.coli*, *K. pneumoniae* and *Acinetobacter*, *Pseudomonas*, *Shigella*, *Salmonella*, *Citrobacter*, *Enterobacter*, etc… This finding reinforces the need for more care with clothing of HCWs.

From a total of 280 isolates *E.coli* (22%) was dominant followed by *Acinetobacter spp* (20.9%) and *S. dysentriae (*11.7%). This was high compared with studies conducted in other African countries (34, 35,70). Many gram-negative bacteria which cause hospital acquired infections such as *Klebsiella spp, Enterobacter Spp, Citrobacter Spp, E. aerogens, Pseudomonas Spp, salmonella Spp, proteus Spp,* P *stuarti, S. marscens and Alcaligens Spp* were identified in this study (Fig:7). Many studies had reported these isolates from gowns of HCWs (35, 63, 66, 67). The high rate of bacterial contamination of white coats may be associated due to patients continuously shed infectious microorganisms in the hospital environment, and physicians are in constant contact with these patients. This has been demonstrated that

different bacteria can survive for around 98 days on environments found in hospitals, such as those used for white coat [81].

Many of gram-negative bacteria isolates had multi drug resistant characteristic or had some resistance to antibiotics tested in this study. Some bacteria had similar antibiotic resistance profile of the same species (e.g., *Acinetobacter Spp, P. mirabilis, Citorbacter Spp, Salmonella Spp*) isolated from different gowns; this could suggests that these could have had a common source of contamination from patients, from contaminated hospital environment or from any unknown source.

Higher resistance pattern was seen for ampicillin within the range of 25%-100% among a total of 13 antibiotics tested. This finding is supported by other studies conducted from white coats of health care workers (21, 61, 63, 67). 49.3% (138/280) isolates were found to have MDR characteristics. A very alarming resistance pattern was recorded among 100% (3/3) *E. aerogens* and 73% (43/59) isolates of *Acinetobacter Spp*. This resistance pattern is in line with study conducted by different researchers (21, 63, 67). Meropenem and imipenem were among the most active antibiotics tested in this study which ranges from 93%_100%. Piperacilin-tazobactam had shown a sensitivity for most isolates but *Acinetobacter Spp* (53%) had resistance compared to the other β-lactam inhibitor amoxicillin –clavulnic acid which shows sensitivity ranges from 50%-100%. Many studies had revealed a similar finding with our current study (82, 83).

It remains unclear as to why the prevalence of ESBL producer from work surfaces, gowns and other inanimate objects was higher than those seen in isolates from other sources. This may be attributable to clonal dissemination of particular ESBL-producing clones, although the confirmation of such supposition is beyond the scope of this study. Previous studies reported that contamination by drug-resistant bacteria may be found on several surfaces, including the floor, the bed frame, the furniture, the patient's clothes and the bed sheets (71, 82-84).

7.0 Conclusion and Recommendation

Conclusion

In the current study ESBL producing gram negative bacteria were isolated both from gowns and mobile phones of HCWs. We have also shown that mobile phones and gowns can be colonized with a variety of pathogenic organisms. The significance of colonization of gowns and mobile phones of HCW and its implications for patient care remains unclear. Mobile phones, gowns and other inanimate objects can lead to bacterial cross-contamination and can be a cause of nosocomial infections, contributing to the spread of resistant hospital infections within the cycle of operating rooms, ICUs, wards, and burn units which are at high risk of acquiring infection.

Recommendation

HCWs should have a habit of routine decontamination of mobile phones with regular cleaning agents to reduce the spread to both internal and external environments. Regulations that specifically mandate good hand hygiene and cleaning mobile phones must be in place to control cross contamination of pathogenic microorganisms.

Gowns protect HCWs from blood borne or other pathogenic infection. But, since gowns are a vehicle and a reservoir for pathogenic microorganisms; we recommended that;

- ✓ Hospital administrators should give extensive attention to raise the awareness on uniforms as a mode of microorganism transmission among all hospital workers.
- ✓ HCW should be advised to wear hospital gowns only in parts of the health care facility where it is needed and strictly avoid wearing them out of these designated areas.

8. References

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Annexes

Annex I: Bacteria Identification Methods

Tentative identification of bacterial cultures was done according to CLSI 2018.

Colony characteristics:

The isolates were preliminarily identified based on morphological characteristics like color, size, margin, form, elevation and texture. .

Gram staining:

Gram staining steps

1. Gently flood smears with crystal violet and let stand for 1 minute.

2. Gently wash with tap water.

3. Gently flood smears with the Gram's iodine mordant and let stand for 1 minute.

4. Gently wash with tap water.

5. Decolorize with 95% ethyl alcohol. Note: Do not over-decolorize. Add reagent drop by drop until the alcohol runs almost clear, showing only a blue tinge.

6. Gently wash with tap water.

7. Counterstain with Safranin for 45 seconds.

8. Gently wash with tap water& blot dry with bibulous paper and examine under oil immersion

Biochemical test for bacterial isolates:

1. Catalase test:

Catalase test (Slide Test)

- ✓ Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
- ✓ Place a drop of 3% H_2O_2 on to the slide and mix.
- ✓ A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.

- ✓ A negative result is no bubbles or only a few scattered bubbles.
- ✓ Dispose of your slide in the biohazard glass disposal container.

2. Oxidase test:

- ✓ A piece of filter paper was divided into three equal section and labeled with the name of organism
- ✓ A loop full of culture was rubbed on the moistened filter paper using a sterile loop
- ✓ The color of the smear was checked exactly 15-30 seconds after rubbing the cells on the reagent moistened filter paper
- ✓ A deep blue color indicates positive reaction
- ✓ Light violet or purple color developed within 10 seconds was recorded as negative.

3. Carbohydrate fermentation and Hydrogen Sulphide production test:

- ✓ Fermentation medium was prepared with specific carbohydrate such as glucose, lactose and sucrose
- ✓ The media was sterilized using autoclave at 151b pressure for 15minutes
- ✓ Each of specified fermentation tubes of media was labeled with the name of the organism to be inoculated
- ✓ The tubes were observed for the change in color (due to production of acid) or changein color and appearance of bubbles (due to production of acid and gas)

4. Indole production and Hydrogen Sulphide production

- ✓ SIM agar medium was prepared and sterilized using autoclave
- ✓ SIM agar tubes were labeled with the name of the organism to be inoculated
- ✓ Each organism were inoculated into its appropriately labeled tubes by means of stab inoculation
- ✓ The tubes were incubated at 35° to 36°C for 48hours
- ✓ The tubes were observed for the presence or absence of black coloration along theline of stab inoculation
- ✓ The tubes were shaken gently after intervals of 10 to 15minutes
- ✓ The tubes were allowed to stand for few minutes to permit the reagent to come to the top
- ✓ The tubes were observed for cherry red layers in the top layer

5. Citrate utilization test:

- ✓ Simmons's citrate agar media was prepared and sterilized using autoclave
- ✓ 5ml of media was poured into the culture tubes and slants were prepared
- ✓ Simmons's citrate agar slants were inoculated with test organism
- ✓ The uninoculated tubes were kept as control
- ✓ The tubes were incubated at 370C for 48hours
- ✓ Slant culture was observed for the growth and coloration of the medium

6. Urease test:

- ✓ Urease agar medium was prepared and sterilized using autoclave
- ✓ The medium was allowed to solidify in the slanting position to form a slope
- ✓ The slants were inoculated with test organism
- ✓ The tubes were incubated at 37°C for 24 to 4hrs
- ✓ The slants were observed for color

7. Mannitol fermentation

- ✓ Select the phenol red mannitol broth medium.
- ✓ Start your Bunsen burner.
- ✓ Select the inoculating loop tool.
- ✓ Flame your inoculating loop to sterilize it.
- ✓ Remove the caps from your test tubes.
- ✓ Flame the mouths of your test tubes.
- ✓ Use the sterile inoculating tool to pick up an inoculum from the culture tube of the unknown bacterium.
- ✓ Immediately transfer the inoculum into the fresh, sterile medium.
- ✓ Flame the mouths of your tubes once again.
- ✓ Replace the caps on the test tubes.
- ✓ Re-flame the inoculating tool.
- ✓ Place the inoculated tube into the 35-37 C incubator.
- ✓ Incubate for the appropriate length of time. For this test, 24 hours is sufficient for determining the result.

8. Malonate utilization test

- ✓ Select the Malonate broth medium.
- ✓ Start your Bunsen burner.
- ✓ Select the inoculating loop tool.
- ✓ Flame your inoculating loop to sterilize it.
- ✓ Remove the caps from your test tubes.
- ✓ Flame the mouths of your test tubes.
- ✓ Use the sterile inoculating tool to pick up an inoculum from the culture tube of the unknown bacterium.
- ✓ Immediately transfer the inoculum into the fresh, sterile medium.
- ✓ Flame the mouths of your tubes once again.
- ✓ Replace the caps on the test tubes.
- ✓ Re-flame the inoculating tool.
- ✓ Place the inoculated tube into the 35-37 C Incubator.
- ✓ Incubate for the appropriate length of time. For this test, 24 hours is sufficient for determining the result.

Annex II: Flow Chart for Gram Negative Rods

	Lactose	Indole	Ur Ea	Man Itol	H_2S	Gas Glu	Citrate	Motility	Lysin	**Organism**	Additional
LACTOSE OR ONPG (ortonitrophnile galactosidase) **POSITIVE** NB:- 1. Ornithine (-) 2. Ornithine (+) 3.Gas variable week. 4.Additional inositol (+) 5.Additional inositol (-)	+	-	+	+	+	+	+	+	-	Citrobactor	Urea +
				+			+	+	-	Entrobactor cloacae	
					-	+		-	+	Klebsiella pneumonia	Malonate +
							-/+	-	-/+	Klebsiella ozenae	
							+/-	+	-	Ent. agglomerans (Erwinia)	
						-	-	-	-	Klebs.rihinoscleromitus1 Shigella sonnei (2)	
					-		+	+	+	Serratia (3)	
			-	+		+	+	+	+	Ent.arogens (4) or Hafnia (5)	
									–	Ent.Cloacae (6) or Citrobactor (7)	
					+	+	+	+	+	Citrobactor	
									+	Arizona	Malonate +
		+	+	+	-	+	+	-	+/-	Klebsellaoxytoca	
							+	+	+	Citrobactordiversus	
				+	-	+	-	+	+	E. Coli	

6.MR$^-$, VP$^+$ 7.MR$^+$, VP$^-$			-					-	+	E. Coli	
						-	-	-	-	E. Coli (A—D)	
				-	-	-	-	-	-	Sh. Dysente or E. Coli A-D	
II	Lactose	Indol	Ur Ea	Ma Itol	H_2S	Gas glu	Cit rate	Mot ility	Lys In	**Organism**	Additional
LACTOSE AND ONPG NEGATIVE NB:- + 90% or more positive -90%or more negative +/- majority negative -/+majority negative	-	**+**		+/-	-	+	+	+	-	Providencia rettgeri	PAD (+)
			+	_	-	+	-	+	-	Morganella morganii	PAD (+) LDC(-)
					+	+	-/+	+	-	Proteus vulgaris	PAD (+)
						+	+	+	-	Providenicaalkalifaciens 4	PAD (+)
				-	-	-	+	+	-	ProvidinicaStuartii	PAD (+)
							-	-	-	Shegelladysentriae	
			-		+	+	-	+	+	Edwardsiella	
							-	-	+	E. coli (A—D)	
				+	-	-			-	Shigella spp.	
							+	+	-	Provideniciastuartii (B)	PAD (+)
		-		-	-	-	-	-	-	Shigella dysentriae	
			-		-	-	-	-	-	Shigella spp.	
				+		+	-	+	_	Salmonella Group A	
					+	+	+	+	+	Salmonella or Arizona	
						-	-	+	+	Salmonella thyphi	VC (+)
			+	-	+	+	+/-	+	-	Proteus mirabilis	PAD (+)
	d	-	D	+	-	+d	+	+	+	Seratiamarcescers	Ox +/-
III	Lactose	Indol	Ur Ea	Ma Itol	H_2S	Gas glu	Cit rate	Mot ility	Lys In	**Organism**	Additional
NON-FERMENTATIVE	-	-	D	+/-	-	-	+	+/-	+/-	Pseudomonas aeruginosa	Cat + Oxi +
	+/-	-	-s	-	-	-	+/-	-	-	Acinetobacter	Cat + Oxi -
	-	-	-	-	-	-	D	+	-	Alcaligens spp.	Cat + Oxi -

Annex III: ESBLs Detection

ESBL will be screened by Double Disc Diffusion Synergy Test (DDST) method with cefotaxime(30µg), and Ceftazidime (30µg), placed 15 mm apart on Mueller Hinton agar from Ceftazidime-clavulanic acid and incubated for 18-20 hours at 35°C-37°C to observe any enhancement zone towards the combination disc. For quality control, the ATCC standard E. coli (ATCC 25922) strain as negative for ESBL production and Klebsiella pneumoniae (ATCC 700603) as positive for ESBL production or equivalent quality control strains will be used.

ESBL positive strain will be measured and interpreted as follows;

Zone diameter for, cefotaxime ≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm for screening

Increase in zone size with addition of inhibitor (ceftazidime+ clavulanic acid) by >5 mm comparing with cefotaxime ≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm alone will be used for confrmation.

List of antimicrobials and their interpretation according to CLSI, 2018

- Maximum of 12 disks on a 150-mm plate and maximum 5 disks on a 100-mm plate.
- Direct colony suspension equivalent to 0.5 McFarland standards Incubation: 35+/- 2 ^{o}c ambient air 16-18 h were applied.

Annex IV: Antimicrobial Susceptibility Testing

Pure bacterial colonies were inoculated into 7 ml of Tryptophan soya broth and incubated at 37 ºC for 18 hours until turbidity is seen and were compared to the 0.5 McFarland standards. Mueller – Hinton Agar was used as plating medium. Fifteen minutes after inoculation of the plates using sterile swabs, the antibiotic impregnated disks were applied on the surface of inoculated plates with sterile forceps. All the disks were gently pressed down onto the agar with forceps. The plates were inverted and then incubated aerobically for 18 hours at 37 ºC. The diameters of the zones of inhibition were measured to the nearest whole millimeter using the transparent ruler and were interpreted as susceptible, intermediate and resistant based on the recommendations of Clinical Laboratory Standards Institute.

AST procedures

1. Disc diffusion method on Muller-Hinton agar (MHA) plates as per CLSI guidelines.
2. Fresh culture from the glycerol stock will be streaked on nutrient agar plates and nutrient agar plates will be incubated at 37 °C for 24 h.
3. Colonies will be transferred into test tube of 5 mL of sterile nutrient broth (or normal sterile saline) adjusted to obtain turbidity matching 0.5 Mc-Farland standards.
4. The isolates will be inoculated onto MHA plates and disks impregnated with antimicrobial agents will be dispensed on the inoculated plates and will be incubated at 37 °C for 18–24 h and zones of inhibition will be measured.
5. Each isolate will be reported as resistant or sensitive to the antibiotics as per CLSI guidelines recommendations (CLSI 2018).

The antimicrobials interpretation of the categories of susceptible, intermediate or resistant will be based on the CLSI guidelines (CLSI, 2018) in annex. Isolates will be regarded as multi-drug resistant (MDR) when they exhibit resistance to at least two or more drugs. E. coli ATCC 25922 will be used as a quality control organism.

(CLSI 2018 breakpoints, when not otherwise specified. CLSI document: M100S27E)

#: A = Always test!
#: AU = Always test, if the specimen is urine! If not urine, replace the disk with another disk in the order of priority (1, 2, 3, etc.)
#: If a disk marked "A" or "AU" is out of stock, or the drug is currently unavailable in the hospital, replace the disk with another disk in the order of priority (1, 2, 3, etc.)
#: If the microbe is sensitive to none or only 1 iv/im antibiotic, test all remaining antibiotics with breakpoints listed for the species
#: Report any antibiotics (add the relevant disks if necessary) that has been or will be administered to the patient (often listed on the request form!), but only if we have breakpoints or interpretative criteria for the antibiotic
#: Note that some antibiotics are listed with "DO NOT TEST". Read "Comment" for guidance on how to report these antibiotics!

Zone Diameter Interpretive Standards for *Enterobacteriaceae*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test = 12
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}c ,ambient air 16-18 hr.

When test?	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ampicilin	10 µg	≤ 13	14-16	≥ 17	Report amoxicillin with S/I/R result from ampicillin
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	Do not report for Salmonella and Shigella spp.
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	Do not report for Salmonella and Shigella spp.
A	Cefotaxime	30 µg	≤ 22	23-25	≥ 26	
A	Ceftriaxone	30 µg	≤ 19	20-22	≥ 23	
A	Ceftazidime	30 µg	≤ 17	18-20	≥ 21	
A	Trimethoprim+Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
A	Ciprofloxacin	5 µg	≤ 20	21-30	≥ 31	

	(breakpoint for Salmonella only)					
A	Ciprofloxacin (breakpoint for non-Salmonella)	5 µg	≤ 15	16-20	≥ 21	
AU	Nitrofurantoin (PO only)	300 µg	≤ 14	15-16	≥ 17	
1	Amoxicillin+clavulanic acid (PO only)	20/10 µg	≤ 13	14-17	≥ 18	
7	Cefuroxime	30 µg	≤ 14	15-17	≥ 18	Do not report for Salmonella and Shigella spp.
4	Cefepime	30 µg	≤ 18	19-24	≥ 25	
5	Cefixime (PO only, only for uncomplicated UTI)	5 µg	≤ 15	16-18	≥ 19	Do not test or report Morganella spp. with cefixime
6	Imipenem or meropenem	10 µg	≤ 19	20-22	≥ 23	If R: Notify leader of Hospital Infection Control Team
2	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	URINE ONLY & only test if ciprofloxacin is N/A
9	Trimethoprim (PO only)	5 µg	≤ 10	11-15	≥ 16	Only test and report for urine isolate (drug N/A))
8	Aztreonam	30 µg	≤ 17	18-20	≥ 21	
3	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18	DO NOT TEST IN URINE. ALWAYS TEST IN CSF.

Zone Diameter Interpretive Standards for *Pseudomonas aeruginosa*, in mm

Testing conditions
Media: Mueller-Hinton agar.
Use maximum 12 disks on a 150 mm plate;
Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.
Number of disks to test: 6
Inoculum: direct colony suspension equivalent to 0.5 McFarland standards
Incubation: 35+/- 2 ^{o}c ,ambient air 16-18 hr.

When test? #	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ceftazidime	30 µg	≤ 14	15-17	≥ 18	
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	
A	Imipenem or Meropenem	10 µg	≤ 15	16-18	≥ 19	If R: Notify leader of Hospital Infection Control Team
A	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
1	Norfloxacin (PO only)	10 µg	≤ 12	13-16	≥ 17	Only test from urine isolates, and only test if ciprofloxacin is not available

2	Cefepime	30 µg	≤ 14	15-17	≥ 18	
	Ceftriaxone	DO NOT TEST	R			Report as R if requested (EUCAST)
	Cefotaxime	DO NOT TEST	R			Report as R if requested (EUCAST)
	Tetracycline	DO NOT TEST	R			Report as R if requested (EUCAST)
	Doxycycline	DO NOT TEST	R			Report as R if requested (EUCAST)

Zone Diameter Interpretive Standards for *Acinetobacter spp*, in mm

Testing condition

Media: Mueller-Hinton agar.

Usemaximum 12 disks on a 150-mm plate;

Use maximum 6 disks on a 100-mm plate. Disks should be placed no less than 24 mm apart, center to center.

Number of disks to test: 6

Inoculum: direct colony suspension equivalent to 0.5 McFarland standards; Incubation: 35+/- 2 ^{o}c ,ambient air 20-24 hr

When test?	Antimicrobial Agent	Disk content	Zone diameter nearest whole mm			Comments
			R	I	S	
A	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21	
A	Gentamicin	10 µg	≤ 12	13-14	≥ 15	
A	Tobramycin	10 µg	≤ 12	13-14	≥ 15	
A	Amikacin	30 µg	≤ 14	15-16	≥ 17	
A	Trimethoprim-Sulfamethoxazole	1.25/23.75µg	≤ 10	11-15	≥ 16	
1	Imipenem	10 µg	≤ 18	19-21	≥ 22	See comment under #
A	Meropenem	10 µg	≤ 14	15-17	≥ 18	See comment under #
7	Ticarcillin	75 µg	≤ 14	15-19	≥ 20	
2	Ceftazidime	30 µg	≤ 14	15-17	≥ 18	
3	Cefepime	30 µg	≤ 14	15-17	≥ 18	
4	Tetracycline	30 µg	≤ 11	12-14	≥ 15	URINE ISOLATES ONLY
5	Doxycycline	30 µg	≤ 9	10-12	≥ 13	
6	Gatifloxacin	5 µg	≤ 14	15-17	≥ 18	
8	Colistin	Gradient MIC	MIC ≥ 4	-	MIC ≤ 2	
9	Tigecycline	Gradient MIC	MIC ≥ 1	MIC = 0.5	MIC ≤ 0.25	”
	Cefotaxime	DO NOT TEST	R			Report as R if requested (EUCAST)
	Ceftriaxone	DO NOT TEST	R			Report as R if requested (EUCAST)

IF MEROPENEM OR IMIPENEM RESISTANT DO THE FOLLOWING: 1: Perform AST for ALL remaining disks and gradient MIC strips in the table 2: Confirm ID of isolate (with API 20 NE, if available). 3. Perform Modified Hodge Test (“clover leaf test”) for the presence of carbapenemase 4. Notify leader of Hospital Infection Control Team

Annex V: Declaration

The undersigned; declare that this study complies with the regulation of the University .This thesis work is my original work and had not been presented in Addis Ababa University or any other university. I also declare that all sources of materials used for the thesis work had been acknowledged. I agree to accept responsibility for provision of required progress reports.

Shambel Araya (BSc)

Signature……

Date of submission……

This thesis had been submitted with my approval as his advisor.

Dr. Yimtubezinash Woldeamanuel (MD, MSc, PhD)

Signature…….

Mr. Kassu Desta (MSc)

Signature…….