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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACTERIAL ISOLATES FROM SEWAGES POLLUTED URBAN RIVERS, ADDIS ABABA, ETHIOPIA, 2017

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List of Abbreviations

AMR----------Antimicrobial Resistance

ATCC---------- American Type Culture Collection

CLSI---------- Clinical Laboratory Standard Institute

DRERC----------Departmental Research and Ethics Review Committee

EPHI---------- Ethiopian Public Health Institute

MDR----------Multi Dreg Resistance

MIC---------- Minimum Inhibitory Concentration

SOP----------Standard Operating Procedure

SPSS----------Statistical Package for Social Science

WHO----------World Health Organization

Abstract

Background: rivers are an important source for drinking, irrigation, recreation and other domestic purposes. However, if it is polluted, it can act as the storehouse of harmful infectious agents that possess multiple drug resistant genes, which is a growing public health concern.

Objective: To determine gram negative bacterial profile and antimicrobial susceptibility pattern from selected sewage polluted urban rivers of Addis Ababa.

Methods: A cross sectional prospective study was conducted from February – April 2017. Water samples were collected from the rivers that pass through ten sub cities of Addis Ababa. Turbid samples were tenfold diluted (1:10) with sterile physiological saline. Samples were inoculated on blood and MacConkey agar. Presumptively isolated organisms were identified by BioMerieux VITEK 2 COMPACT machine. Antimicrobialsusceptibility patternwas alsoperformedfor 19 antibiotics by the machine with minimum inhibitory concentration technique. Epidata 3.1 was used as data entry and SPSS 20 was used for data analysis.

Results: From a total of 94 river water samples, 150 gram negative bacterial isolates were recovered to the species level and 30 isolates were not identified by the machine. The isolation rate was 98% (92 samples were positive for one or more than one bacterial isolates). The predominant species were *A. hydrophylia/cavae* 26 (17%) followed by *E.coli* 23(15%), *K.pneumonia* 18(12%), *Rautella* species 18(12%), *K. oxytoca* 17 (11%).The least identified species was *E.gergovia and C.braki*1(1%). *A. Hydrophila/Cavae* showed high level of resistance to cefazolin 10 (38%), cefoxitin 9 (35%) and trimethoprim sulphomethoxazole 10 (38%). *E.coli.* showed also high level of resistance to many of tested antibiotics like ampicillin 21 (91.3%), and 16(70%) resistance to Cefalotin, Cefuroxime, Ceftriaxone and Cefepime. Both *K.Pneumoniae* and *K.oxytoca* showed high resistance to ampicillin 16(94%) and 17(95%) respectively. Among identified bacterial species, most of them showed multidrug resistant pattern. *Providential retigerri* showed 100% MDR Followed by *P.Alkalificiens* (90%), *E.coli* (78%), *Morganella* species (75%), and *C.frundi* (60%).

Conclusion and Recommendation: *A.hydrophila/cavae* and *E.Coli*were the most predominant bacterial isolates. Most of identified bacterial species were highly resistant to ampicillin and amoxicillin/clavulnic acid. Drug resistance and MDR were very high in this study.

Key words: Gram negative bacteria, Multidrug Resistance, Antimicrobial susceptibility pattern, River, Addis Ababa

1. Introduction

1.1. Background

Water is considered a vehicle for the propagation and dissemination of human associated bacteria (1). Safe drinking water is a fundamental human right and if contaminated with opportunistic pathogenic environmental bacteria, it may have health implications for consumers (2). Human health should therefore be protected by preventing microbial contamination of water that is intended for consumption (3). In rural communities, untreated surface water from rivers, dams, and streams is directly used for drinking and other domestic purposes (4). These unprotected water sources can be contaminated with microbes through rainfall runoff, agricultural inputs, mixing with sewage effluents and faeces from wild life and health care institutions, which render them unacceptable for human consumption (5).

The use of antimicrobials in human and animal health care has resulted in the widespread prevalence of antimicrobial resistant (AMR) bacteria not only in humans and animals, but also in the environment, e.g. in surface water and soil (6, 7, 8, 9).

The majority of antibiotics used are partially metabolized after administration and released through patient excreta into the municipal sewage system. Untreated liquid waste containing partially metabolized antibiotics in low concentration contributes largely to the development of antibiotic resistance in our natural/environmental micro flora (10, 11, 12, 13). Therefore, if the hospital and municipal sewage effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed into rivers and finally reach to communities resulting in water borne diseases such as cholera, typhoid fever, dysentery and gastroenteritis which cannot be treated by conventional antibiotics(14).

Dissemination of bacteria with acquired resistance to antimicrobials may represent both direct and indirect risks to human health. The direct risk entails exposure to AMR pathogens, resulting in hard to treat infections. Indirect risks are associated with the exposure to relatively harmless AMR bacteria, such as commensal bacteria that are able to colonize gut, skin or mucosa, resulting in asymptomatic carriage of AMR bacteria (15). The public health risks associated with asymptomatic carriage comprise transfer of resistance genes between commensals and pathogens, transfer of AMR commensals (that are often opportunistic

pathogens) to people who are more vulnerable to infection or asymptomatic carriers entering a stage of increased vulnerability themselves (e.g. hospitalization) (16).

Infection with MDR bacteria is another big issue associated with hospital effluents and it carried simultaneous resistance to 10 or more routinely used antibiotics and could pose a serious threat tothe community (17). The occurrence of strongly selective environments for antimicrobials, such as hospitals, promotes, not only the growth of resistant bacteria, but also leads to an increase in the frequency of resistance bacterial genes and genetic elements such as plasmids, as the result, MDR bacteria could transfer their drug resistance genes to sensitive pathogens. This in turn could cause infections in the community difficult to treat. The reported detection rate of Salmonella and *Shigella* species in hospital wastewater effluents was high and could pose a serious threat to community health (18).

Even if both gram negative and gram positive bacteria are involved for development of wastewater associated disease, gram-negative bacteria such as *E.* coli, *Salmonella* species, *Shigella* species, *Klebsiella* species and *Enterobacter* species are the most crucial in this aspect because they are the most common causes of hospital and community acquired infections and they are inherently resistant to many hydrophobic antibiotics (19).

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1.2. Statement of the Problem

Poor health care and domestic waste management in the developing countries is the major cause and problem of the society and their environment. Though, there are different mechanisms to manage healthcare wastes, developing countries including Ethiopia faced challenges in managing health care wastes particularly hazardous wastes which might seriously affect health of the society (20).

The spread of MDR among pathogenic and commensal bacteria is a global health concern. Each year about 17 million people die from infectious diseases worldwide, most of which are caused by bacteria (21). The Centers for Disease Control and prevention (CDC) has stated that in the United States more than two million people are sickened and 23,000 die each year as a result of antibiotic-resistant infections (22). Antibiotic resistance can lead to increases in human and domestic animal health care costs as well as increased mortality and morbidity (23). Consumption of contaminated water or food consequently leads to gastrointestinal tract infection by various bacteria including *E. coli* (24).

Antimicrobial resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections and a common cause of both community and hospital acquired bacteremia. Moreover, the transfer of antibiotic resistance determinants from *E. coli* to normal flora of gastrointestinal tract has been reported (25). Due to the fact that consumption of contaminated food has been considered the main route of transmission of drug resistance, relatively little attention has been paid to other routes by which antibiotic resistance can be disseminated, such as natural water, wastewater and soil environments (26).

Recently, in its first global report on surveillance of antimicrobial resistance, the World Health Organization (WHO) reported very high rates of resistance in bacteria (e.g., *E. coli, K. pneumoniae*, and *S. aureus*) that cause common healthcare-associated and community-acquired infections (27).

The World Health Organisation (WHO) alsoestimates that about 1.1 billion people globally drinkunsafe water and the vast majorityof diarrhoeal disease in the world (88%) is attributabletounsafewater, sanitation and hygiene. Approximately 3.1% of annual deaths (1.7 million)and 3.7% of the annual health burden world-wide (54.2 million)are attributable to unsafe water, sanitation and hygiene. In 2002, diarrhoea contributed 4.3% of the global

disease burden, of which 88% is caused by unsafe water, sanitation, and hygiene (19). In the USA, 9% of all outbreaks with the pathogenic E. coli O157 are waterborne (28).

As Addis Ababa is the capital city of Ethiopia and the destination for Africa, with population of more than 3,384,569 (29), health issue related to hospital effluent should not be underestimated. According to ministry of health, Addis Ababa has more than 39 Hospitals Run by the Ministry of Health and Private Entities (MoH 2006/07) and 26 health centers, 507 clinics and 42 health posts (Some Health Service Information, Addis Ababa Bureau of Health, 2004/05, Un published). Like other developing countries, most hospitals and other health care institutions in Ethiopia, do not have wastewater treatment plants, which worsens the problem. Finally, the untreated wastewater from hospitals and municipal sewages will join rivers, streams or disposed to underground water and people will use for drinking, domestic, irrigation and recreational purpose. Particularly, rivers are one of the major sources of water directly or indirectly for human and animal consumption, its pollution may contribute to the maintenance and even the spread of bacterial antibiotic resistance (30).
In the study area, there is no any research done on bacterial profiles and antimicrobial susceptibility patterns from rivers. So the aim of this study is to assess bacterial profile and microbial susceptibility pattern of bacteria in polluted urban rivers, Addis Ababa Ethiopia.

1.3. Significance of the Study

- ✓ This study will alarm policy makers to manage hospital effluents and municipal sewages from discharging to rivers.
- ✓ Information from routine susceptibility testing of bacterial isolates will be important for physicians and other stakeholders to update themselves on resistance trends, including emerging antibiotic resistance for clinical practice.
- ✓ It will also be used as baseline information for further study in the study area.

2. Literature Review

Microbiological contamination is an important water-quality problem worldwide. Human impact on this category of contamination is significant and several human-related activities, and also the population explosion, have affected and are still affecting dramatically the aquatic environment. Selection and dissemination of resistant bacteria in nature should be avoided in order to ensureeffective treatment against infectious disease in humans and maintain an ecological balance thatfavours the predominance of a susceptible bacterial flora in nature (5).

Large quantities of antibiotics are used in hospitals for patient treatment. Most of the antibiotic taken by the patients are partially metabolized and excreted through feces and urine. After use, residual quantities of these products reach the wastewater, exposing the bacteria that survive in hospital wastewaters to a wide range of biocides that could act as a selective pressure for the development of resistance (14). Increasing attention has been directed recently to the resistance of bacteria to antibiotics and disinfectants. The resistant bacteria isolated were diverse in nature. For example, study conducted in Buenos Aires City hospital, Brazil, the bacterial population resistant to disinfectants was mainly composed by Enterobacteriaceae, Staphylococcus spp, and Bacillus spp, which are highly associated to nosocomial infections (16).

Co-resistance to antimicrobial agents among organisms were also reported in different studies and an indication of the risks posed by the untreated effluents to public health as well as increasing evidence about the role of hospital wastewaters as environmental reservoir of multi-drugresistant bacteria(17).For example, study conducted in Netherland on Multidrug-Resistant and Extended Spectrum Beta Lactamase-Producing Escherichia coli showed that a total of 846 E. coli isolates were recovered from 113 surface water samples and the proportion of *E. coli* isolates resistant to at least one class of antimicrobials (AMR E. coli) varied from 7% to 49%. 26% of isolated *E. coli* were AMR, and 11% were MDR (31).

Co-resistance to antimicrobial agents among organisms are reported in different studies and an indication of the risks posed by the untreated effluents to public health as well as increasing evidence about the role of hospital wastewaters as environmental reservoir of multi-drugresistant bacteria. The study in Romania showed that from eight (8) of surface (river) water samples, both non enteric and enteric bacterial strains were isolated. Among

enteric isolated bacterial species, *E.coli*, *Klebsella*, *Enterobacter* and *Citrobacter* species were the the most predominant. Those Gram-negative bacterial strains showed very high levels of resistance to AMP (63.07%), AMC (54.87%) and high levels of resistance to KZ (49.74%), and FOX (27.69%). Resistance to third generation cephalosporins was intermediate for CTX (18.97%) and CRO (17.44%) and low for CAZ (9.23%). Resistance to other classes of antibiotics (non beta-lactams) was high to folate inhibitors (SXT, 31.79%), intermediate to tetracyclines (TE, 19.48%), aminoglycosides (NN, 16.92%) and fluoroquinolones (CIP, 10.76%). High resistance rates to TE (43.01%) and NN (29.31%) was also found in *E. coli*. High multidrug resistance rates were registered in *E. coli* (60.34%), *Enterobacter* (34.38%) and *Klebsiella*species (32.76%) (32).

The study done in Poland on Antibiotic resistant *E.coli* showed that from 21 river samples, 38 *E.coli* strains were discovered. Almost all (95.7%)*E. coli* strains proved to be resistant to cefotaxime. Resistance to other cephalosporins (ceftazidime) and aminoglycosides (gentamicin, tobramycin and amikacin) ranged from 21.1% to 94.7% were also reported. Over 61% and 48% of isolates were resistant to the standard doses of gentamicin and amikacin, respectively. 18.4% were resistant to pencillin antibiotics (33).

In Austria the study was done on antimicrobialresistance investigations from sewage, sludge and Receiving Rivers. A total of 767 *E. coli* isolates were tested regarding their resistance to 24 different antibiotics. Among the antimicrobial agents tested, the highest resistance rates in the penicillin group were found for Ampicillin (18%) and Piperacillin (12%). In the cephalosporin group, Cefalothin accounted (35%) and Cefuroxime-Axetil (11%). Trimethoprime-Sulfamethoxazole and Tetracycline accounted (13%) and (57%) resistance respectively (34).

Another study in Mexico was done on 23 river water samples and a total of 33 Gram negative isolates were recovered. Results revealed that all 33 belonged to the *Enterobacteriaceae*, *Pasterurellaceae*, *Vibrionaceae*, and *Moraxellaceae*families. *A. hydrophila*, *E.cloacae*, *E. coli*, *Hafnia alvei*, K. *oxytoca*, *K.pneumonia*, *C. freundii*, *Salmonella* species, S.*enterica* subspecies *Enterica* were among commonly identified *Enterobacteriaceae*. All 33 isolates were susceptible to gentamicin, imipenem, meropenem and tobramycin. However, 39.4% of isolates were resistant to ampicillin; 36.4% of isolates were resistant to cefazoline; 21.2% of isolates were resistant to ampicillin-sulbactam; 12.1% of isolates were resistant to aztreonam,

cefepime, cefotaxima, cefuroxime and trimethoprim-sulfamethoxazole. MDR were identified as *K,oxytoca* (two out of four identified isolates), *E. coli* (2/7), and *E. cloacae* (1/3) (35).

In Portugal another study was done on 12 rivers, and 151 Gram-negative strains were isolated *Aeromonas* species, *E. coli*, *Acinetobacter* species, Pseudomonas species, *Enterobacter*species *C. freundii* and *Alcaligenes* species were among identified strains. Antibiotic resistance profiles showed Resistance to tetracycline was significantly high 26 (17.3%) of the total isolates. 55% of the isolates showed resistance to sulfamethoxazole-trimethoprim. 117 (77.5%) of the total isolates were multidrug resistant (36).

In Brazil similar study was done on river water and *Pseudomonas, Acinetobacter, Aeromonas,* Enterobacteriaceae and Bacillus were detected. Among the strains, many resistances were found, particularly to furazolidone, ampicillin and streptomycin. Little resistance was also found to tetracycline and, except for *Acinetobacter*, to trimethoprim-sulphamethoxazole and to chloramphenicol (37).

The study in Spain showed that from 6 river water samples, A total of 228 bacterial strains were isolated and 110 enterobacterial strains (23 *E. coli*, 23 *Enterobacter*, 22 *Klebsiella*, 19 *Kluyvera*, 15 *Citrobacte*r, 3 *Serratia*, 3 indole-positive *Proteus*, and 2 *Yersinia frederiksenii*) and 118 *Aeromonas* strains (88 *A. caviae*, 19 *A. sobria*, and 11 *A. hydrophila*). Most of the enterobacteriacae (58.2%) showed resistance to the antibiotics tested. Similar resistance rates were observed for nalidixic acid (20%) and tetracycline (18.2%). However, all strains were uniformly susceptible to aminoglycosides. Multiple-antibiotic-resistant enterobacteriacae (isolates with more than three acquired resistances) were uncommon (5.5%). Among the *Aeromonas* strains, 75% showed at least one acquired resistance. Nalidixic acid resistance (72%) was much more frequent than tetracycline (21%) or co-trimoxazole (14%) resistance. Resistances to other antimicrobial agents (chloramphenicol, fosfomycin, beta-lactams, and aminoglycosides) were scarce (5%). Multiple-antibiotic-resistant *Aeromonas*strains were rare (3.4%) (38).

In South Africa the study was done on river and fountain water and a total of 201 bacterial isolates were identified. *E.coli* (40) was the predominant bacteria followed by *Salmonella* (30), *Shigella* (30) *Campylobacter* (26) and *Aeromonas, enterobacter,* and *Plesiomonasshigelloides* species each accounted 20 %. The list prevalent bacteria was vibrio (15%). Antibiogram results of bacterial enteropathogens revealed marked susceptibilities

(over 90%) of *Campylobacter*, *Salmonella* and *E.* coli to nalidixic acid, ciprofloxacin and amikacin. All (100%) of *Salmonella* species were susceptible to Amikacin. 88% of *Campylobacter* species and over 90% of *E. coli*, *Salmonella, Plesiomonas* and *Shigella* species were susceptible to gentamicin. *Aeromonas*, *Enterobacter* and *V. cholerae* demonstrated over 90% susceptibilities to ciprofloxacin and over 85% of the three isolates were in addition susceptible to amikacin. In general, over 80% of all bacteria isolated were susceptible to nalidixic and gentamicin, ciprofloxacin, amikacin and ceftiriazone. At least 20% of bacterial isolates demonstrated antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphericol. A total of 35% of the *Campylobacte*r isolates were resistant to erythromycin whereas 20% to 30% of Campylobacter, Salmonella, *Shigella,Plesiomonas, Aeromonas* and *Enterobacter* isolates showed resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol. Results presented showed multiple antibiotic resistance of all bacteria isolates to ampicillin, erythromycin, tetracycline, chloramphenicol and cotrimoxazole (39).

Another study was done in Nigeria on large river water samples (180) and five types of bacterial isolates (*Bacillus, Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* species) were recovered. 100% resistance to antibiotics was found in *Micrococcu*s species and *Pseudomonas* species while another *Micrococcus, Streptococcus, Staphylococcu*s and *Bacillus* species showed between 40% and 90% resistances. Multiple antibiotic resistance (MDR) was shown by all the isolates and *Bacillus, Micrococcus* and *Pseudomonas* spp. showed the highest resistances (100%) to all antibiotics (40).

The study in Egypt on antibiotic resistance profiles in *Enterobacteriaceae* family isolates from River Nile showed that a total of 293 enteric bacterial isolates were recovered from fifteen sites of sample collection. Among the isolates, 187 (63.8%) were *E. coli*, 59 (20.1%) *Proteus vulgaris*, 25 (8.5%) *S. typhi* and 22 (7.5%) *C. freundii*. All *Enterobacteriaceae* isolates exhibited 100% resistance to Ampicillin, Carbenicillin, Methicillin, Vancomycin, Erythromycin, Clindamycin, Trimethoprim/Sulfamethoxazole and Tetracycline. Multiple antibiotic resistance (MAR) in enteric bacterial isolates from all the sites were detected (41).

Even if many researches done in the world on determining AST patterns of gram negative bacterial species, still it is a challenge, especially for developing countries. In Ethiopia, researches on gram negative bacterial isolates and their susceptibility pattern from rivers

water is limited. Therefore; this study aimed to determine isolation and resistant pattern of gram negative bacterial isolates from river water.

3. Objective of the Study

3.1. General Objective

To determine gram negative bacterial profile and their antimicrobial susceptibility pattern from selected polluted urban rivers of Addis Ababa, Ethiopia.

3.2. Specific Objective

- To identify gram negative bacteria in polluted urban rivers
- To determine bacterial isolates susceptibility pattern to different class of commonly used antibiotics

4. Materials and Methods

4.1. Study Area and Setting

The study was conducted on selected polluted urban rivers that passes through ten sub cities of Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia, with a population of 3,384,569 with annual growth rate of 3.8% (29).

4.2. Study Design and Period

A cross sectional study design was employed from February -April, 2017

Population

4.2.1. Source of Population

All rivers in 10 sub cities were used as a source of population.

4.2.2. Study Population

Rivers that have large water streams (flow) in 10 sub cities were study population.

4.3. Variables of the Study

4.3.1. Dependent Variables

- Gram negative bacterial profile
- Antimicrobial susceptibility pattern

4.3.2. Independent Variables

- ✓ Location of rivers

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria

Riversthat have large water stream were included as study subjects.

4.5. Sampling Methods and Procedures

Rivers that have large water streams (flow) were selected from each sub cities of Addis Ababa. About 32 rivers were included for this study. Grab sampling technique was employed for sample collection. From 10 rivers, 3 discrete samples (1from first sampling point,1 from 100m downstream and 1 from 200m downstream) were collected from each river in first round and processed independently. In the second round, two discrete samples (1 from firs sampling point and 1 from 100 downstream) were collected and processed independently. From the remaining 22 rivers a single discrete sample was collected from each river in the first and second round. Second round samples were collected with 15 days interval.

A total of 94 river samples (12 from Gullele, 8 from Addis Ketema, 12 from Arada, 10 from Lideta, 8 from Kolfe, 11 from Yeka, 8 from bole, 8 from Kirkos, 7 from Nifas Silk and 10 from AkakiKality) were taken. All samples were manually collected by using actual sample containers, then transported immediately to EPHI Microbiology Laboratory with cold chain for further processes within four hours of collection. A clean pair of new, non-powdered, disposable gloves were used each time while collecting water samples from different rivers of ten sub cities. Suitable protective clothe was worn during sample processing to avoid personal contamination. About 150 ml sterile glass containers were used to collect 100 ml wastewater samples.

4.5.1. Sample Processing, Isolation and Identification of Bacteria

Turbid wastewater samples were tenfold serially diluted (1:10) with sterile physiological saline and then 5-20 µl of aliquot was used to streak a single media by using 1µl sterile plastic inoculating loop on blood and MacConkey(Oxoid,UK) agar and incubated aerobically at 37°C for 24–48 hours (43). On the following day, based on colony morphology and other important features such as fermentation on MacConkey agar, metallic sheen and swarming properties on blood gar plate, colonies from primary media were sub cultured to get pure colonies by touching a single colony with sterile plastic inoculating loop (44, 45, and 46). Presumptively isolated organisms were further identified to species level by BioMerieux VITEK 2 COMPACT machine. Once species identification was done by the machine, confirmed species were also tested for 19 antibiotics. Results of antimicrobial susceptibility tests was interpreted based on clinical laboratory standard institute (CLSI) guide line 2015 (47).

The multidrug-resistance (MDR), was defined and interpreted based on interim standard definitions for acquired resistance published in Clinical Microbiology and Infection and formulated by a group of international experts (48).

4.5.2. Principle of Biomerieux VITEK 2 Compact Machine for Species Identification and Antimicrobial Susceptibility Pattern

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The VITEK 2 compact format focuses mainly on industrial microbiology testing environment. It has also great application to clinical laboratories. The machine basically designed based on identification of bacterial isolates and their antimicrobial susceptibility patterns by using colorimetric reagent identification cards (ID- GN) which used for gram negative bacteria identification. Those cards mainly have 64 wells that can each contain impregnated individual test substrate which measures metabolic activities of bacterial isolates such as acidification, alkalinisation, enzyme hydrolysis and growth in the presence of inhibitory substances.

For AST determination, the AST card (GN-AST-72) is working based on MIC technique reported by Maclowry and Marsh(49). The AST card essentiallyuses a double micro dilution technique (50). Each test card contains 64 micro wells. A control well,that contains only microbiological culture medium, resident on all cards, with the remaining wells containing premeasured amounts of specific antimicrobials combined with culture medium.

4.5.3. Test Procedure for Gram Negative Bacteria Identification and Antimicrobial Susceptibility Testing

Each organism suspension was prepared from the growth of pure cultures of bacteria cultivated on plates containing Maconkey agar (Oxoid,UK) and incubated overnight at 35-37°C. A sufficient number of colonies from a pure young culture was transferred to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% NaCl, pH 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube and agitated well till it become homogeneous suspension. The turbidity of the solution was adjusted to 0.5 Mc Farland standard and measured using a turbidity meter called the DensiChekTM (bioMérieux). Once the suspension prepared, 145µl of the suspension was transferred to another similar polystyrene plastic test tube which contain 3 ml of 0.45% Nacl solution by specifically designed pipette to gram negative bacteria and agitated. Test tubes containing the microorganism suspension was placed into a special rack (cassette) and the identification card (ID-GN for identification and AST GN-72

for antimicrobial susceptibility testing) was placed in the neighbouring slot while inserting the transfer tube into the corresponding suspension tube. The cassette accommodated 10 tests. Barcodes from each test card was read by barcode reader and each specimen in the test tube was labelled to differentiate one from the other. Then the filled cassette was manually placed into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the bacterial suspension is forced through the transfer tube into micro-channels that fill all the test wells. After card wells were inoculated, cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types are incubated on-line at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period. A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or coloured products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present. Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database. “Un identified”,”slash line”,”non-reactive”and low discrimination are among possible results if the bacterial isolate does not correctly identify to the species level.

4.6. Quality Control

The reliability of the study findings was guaranteed by implementing Quality control measures throughout the whole processes of the laboratory works. All materials, equipment and procedures were adequately controlled. Aseptic techniques were followed in all the steps of sample collection and Biosafety Cabinet 2B was used during inoculation onto culture media to minimize contamination. All culture media were prepared according to the directions of the manufacturers. The sterility of culture media were assured by incubating 5% of each batch of the prepared media at 35-37 ºC for 24 hours. Performance (selectivity and differentiation) of MacConkey agar was checked by inoculating ATCC strain of *E. coli*

(ATCC 25922), *E. fecalis* (ATCC 29212) and *P.mirabilis* (ATCC 35659). Performance of blood agar was checked by inoculating *S.pneumonia* (ATCC 49619), *S.pyogense* (ATCC 19615) and *E.Fecalis (*ATCC 29212). Pre-analytical, analytical and post-analytical stages of quality assurance that were incorporated in standard operating procedures (SOPs) of EPHI were strictly followed.

4.7. Data Analysis

Datawasenteredintoepi data3.1andexportedto SPSSversion20 for analysis.Descriptive and summary statistics were done. Patterns of quantitative values were presented using statistical tables.

4.8. Operational definitions

Multidrug Resistance: concomitant resistance to one or more antimicrobial agents of three or more classes.

Sewage: Sewage is wastewater that often contains faeces, urine and laundry waste formed by domestic households, industrial and agricultural practices.

Polluted River:A large natural stream of water polluted with both organic and inorganic wastes discharged from domestic health care sewerages.

Grab sample: samples collected from a single sampling point at a time and processed independently

4.9. EthicalConsideration

Ethical clearance was obtainedfrom department of research ethicalReview Committee (DRERC) of Medical LaboratoryScience,Schoolof Allied Health Science, CollegeofHealthScience,AddisAbabaUniversity and Addis Ababa water and sewerage authority. Permission were obtained from administrators of sub cities.

3.11Result Dissemination

Thecopy ofresultwillbedisseminatedtorelevantauthoritiesandtowhomthatneed theinformationforfurtherstudy.Thefindingwillalsobepresentedtothe medicalscientificcommunity andmanuscriptwillbesubmittedtopeer-reviewedjournalsfor publication.

5. Results

5.1. Pattern of Bacterial Isolates

A total of 180 gram negative bacterial isolates were recovered. Among them,150 isolates were further identified to the species level and the remaining 30 were not confirmed to the species level.The most frequent species were *A. hydrophylia/cavae*accounts 26 (16%) followed by *E.coli* 23(15%), *K.pneumonia* 18(12%), *Rautella*spp 18(12%), K.oxytoca(11%). The least identified bacterial species were*E.gergovia* and *C.braki*(1%each)(Table 1).

Table 1: Frequency of Gram Negative Bacterial Isolates from Urban Rivers, June, 2017, Addis Ababa

Bacterial isolates	**Frequency**	**Percent (%)**
A. hydrophila/cavae	26	17
E. coli	23	15
K. pneumoniae	18	12
Raultella species	18	12
K.oxytoca	17	11
P. alkalificiense	10	7
C. Fundi	10	7
P. rettigeri	8	5
A. Hydrophylia	5	3
Morganella species	4	3
A. Sobria	3	2
E. Clocae	3	2
K.Cryovcresence	*3*	*2*
C. Braki	1	1
E. Gergovia	1	1
Total	**150**	**100%**

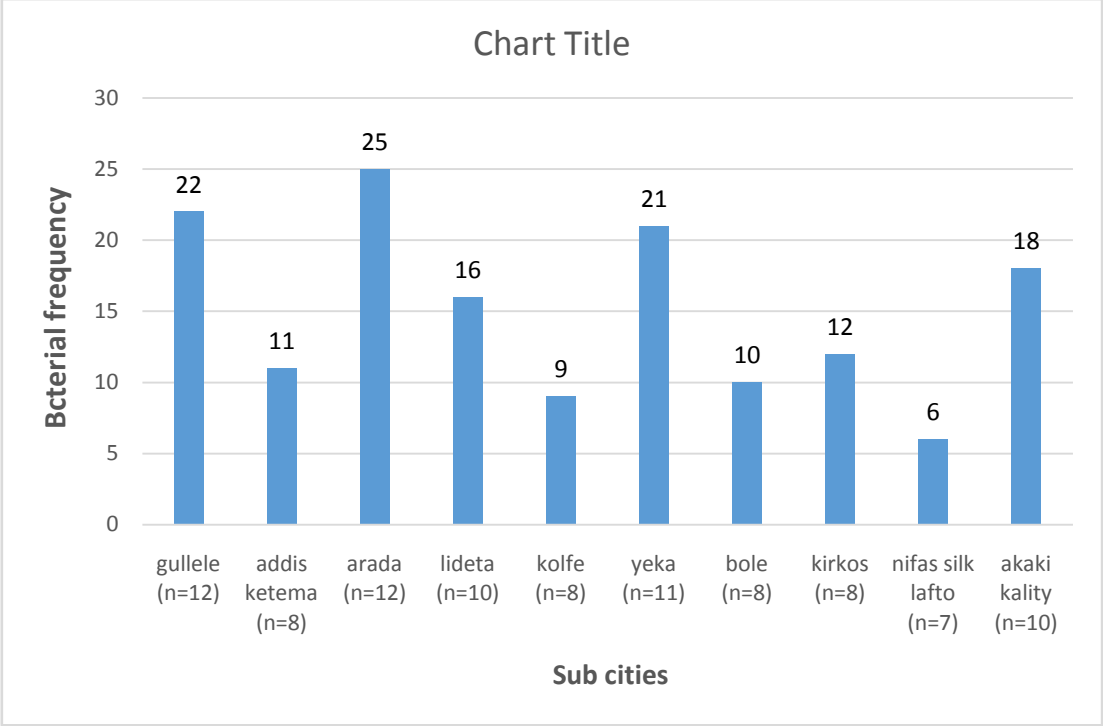

Figure 1: Bacterial isolate frequency among sub cities of Addis Ababa

5.2. Antimicrobial Susceptibility Patterns of Bacterial Isolates

The predominant isolate, *A. hydrophila/cavae*26 (17 %),showed high level of resistance to cefazolin 10 (38%), cefoxitin 9 (35%) and trimethoprim/sulfamethoxazole 10 (38%). However, it showed very high sensitivity to levofloxacin, ceftazidime, tobramycin or gentamicin and ciprofloxacinwith rate of 96 %, 92 %, 89%, and 85% respectively. The second predominant gram-negative isolate, *E.coli*, showed high level of resistance to many of tested antibiotics like ampicillin 21 (91 %), cefalohtin, cefuroxime, ceftriaxone, cefepimeeach accounted 16(70%). However, it was highly sensitive to nitrofurantoin. 17 (74%), tobramycin 14(61%) and levofloxacin 11(47%). Both *K.pneumonia*17(95%) and*K. oxytoca*16(94%) showed high resistance to ampicillin. However, both *K.pneumonia*15(83%) and K. *oxytoca*15(88%) species showed high sensitivity to levofloxacin. Another bacterial isolate, *C. frundi* showed very high resistance to cefazolin,amoxicillin-clavulnic acid and cefuroxime; 8 (80%), 7 (70 %), 7 (70%) respectively. 2(67%) of *E.clocae* showed high resistance to cefalothin,amoxicillin-clavulnic acid,cefazolin, cefuroxime, cefuroxime axetil and cefoxitin. However, all isolates were 100% sensitive to ceftriaxone, tetracycline,nitrofurantoin and trimethoprim-sulfamethoxazole(table 2)

Table 2: Antimicrobial Susceptibility Patterns of Gram-Negative Bacterial Isolates from river water, June, 2017, Addis Ababa Ethiopia

Anti-microbial	Bacterial Isolates														
	E.coli (No. =23)			*K.pnumonia*(No.=18)			*K.oxytoca* (No.=17)			*C.frundi*(No.=10)			*C.braki*(No.=1)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AM	2(9)	0(0)	21(91)	1(6)	0(0)	17(94)	0(0)	1(6)	16(94)	0(0)	1(10)	3(30)			
AMC	13(57)	1(4)	12(52)	14(78)	2(11)	2(11)	12(71)	2(12)	3(18)	3(30)	0(0)	7(70)	0(0)	0(0)	1(100)
TZP	11(48)	1(4)	10(43)	16(89)	0(0)	2(11)	13(76)	1(6)	3(18)	4(40)	0(0)	4(40)	1(100)	0(0)	0(0)
CF	6(26)	1(4)	16(70)	14(78)	1(6)	3(17)	9(53)	2(12)	6(35)	2(20)	0(0)	6(60)	0(0)	0(0)	1(100)
CZ	8(35)	1(4)	14(61)	15(83)	0(0)	3(17)	12(71)	0(0)	5(29)	1(10)	1(10)	8(80)	0(0)	0(0)	1(100)
CXM	6(26)	0(0)	16(70)	13(72)	0(0)	5(28)	11(65)	0(0)	6(35)	2(20)	0(0)	7(70)	0(0)	0(0)	1(100)
CXMAX	6(26)	3(13)	14(61)	12(67)	0(0)	6(33)	11(65)	1(6)	5(29)	3(30)	1(10)	5(50)	0(0)	0(0)	1(100)
FOX	10(43)	1(4)	10(43)	14(78)	1(6)	3(17)	14(82)	0(0)	3(18)	4(40)	1(10)	4(40)	0(0)	0(0)	1(100)
CPD	5(22)	0(0)	17(74)	13(72)	0(0)	3(17)	12(71)	0(0)	5(29)	5(50)	0(0)	3(30)	1(100)	0(0)	0(0)
CAZ	7(30)	1(4)	15(65)	13(72)	1(6)	3(17)	11(65)	0(0)	6(35)	8(80)	0(0)	2(20)	1(1000	0(0)	0(0)
CRO	5(22)	0(0)	16(70)	13(72)	0(0)	4(22)	11(65)	0(0)	6(35)	6(60)	0(0)	4(40)	1(100)	0(0)	0(0)
FEP	6(26)	1(4)	16(70)	13(72)	0(0)	5(28)	11(65)	0(0)	6(35)	6(60)	0(0)	4(40)	1(100)	0(0)	0(0)
GM	11(48)	2(9)	10(43)	15(83)	0(0)	3(17)	13(76)	0(0)	4(24)	7(70)	0(0)	3(30)	1(100)	0(0)	0(0)
TM	14(61)	0(0)	9(39)	13(72)	1(6)	4(22)	13(76)	0(0)	4(24)	7(70)	0(0)	2(20)	1(100)	0(0)	0(0)
CIP	10(43)	1(4)	12(52)	14(78)	1(6)	3(17)	13(76)	2(12)	2(12)	6(60)	1(10)	3(30)	1(100)	0(0)	0(0)
LEV	11(48)	2(9)	8(35)	15(83)	0(0)	3(17)	15(88)	0(0)	2(12)	8(80)	0(0)	1(10)	1(100)	0(0)	0(0)
TE	9(39)	0(0)	14(61)	11(61)	1(6)	6(33)	13(76)	1(6)	3(18)	6(60)	0(0)	4(40)	1(100)	0(0)	0(0)
FT	17(74)	1(4)	4(17)	9(50)	5(28)	3(17)	14(82)	2(12)	1(6)	6(60)	3(30)	1(10)	1(100)	0(0)	0(0)
SXT	10(43)	0(0)	13(67)	13(72)	0(0)	5(28)	11(65)	0(0)	6(35)	6(60)	0(0)	4(40)	1(100)	0(0)	0(0)

Key ***R=Resistant*** ***I= Intermediate Sensitive*** ***S= Sensitive***

AM =Ampicillin
AMC=Amoxicillin/Clavulnic acid
TZP = Piperacillin/Tazobactam
CF = Cefalothin
FEP= Cefepime
GM= Gentamicin
TM =Tobramycin
CZ= Cefazolin
CXM = Cefuroxime
CXMAX= Cefuroxime Axetil
FOX= Cefoxitin
CIP= Ciprofloxacin
LEV= Levofloxacin
TE= Tetracycline
CPD = Cefpodoxime
CAZ= Ceftazidime
CRO= Ceftriaxone
FT= Nitrofurantoin
SXT = Trimethoprim/Sulfamethoxazole

1. *IF S, I and R cells are Blank in the table, it shows that the machine did not test AST patterns of that particular antimicrobials for all number of bacteria mentioned on the same column.*
2. *If only S or I or R is written among the three options (S, I, R), it shows that only AST pattern of that particular antimicrobial is done for one of the isolates found on the same column.*
3. *If the sum of S, I and S is not equal to number of isolates (100%), it should be considered as AST test did not done for some of the species for that particular antimicrobial.*

Table 3: Antimicrobial Susceptibility PatternsContinued…….

Antimicrobias	Bacterial isolates														
	*Raoutella*species (No= 18)			*Morganella* species (No = 4)			*K.ryovcresence* (No = 3)			*E.clocae*(No=3)			*E.gergovia* (No=1)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AM	0(0)	4(22)	14(78)	1(25)	0(0)	3(75)	0(0)	1(33)	2(67)			1(100)			
AMC	15(83)	2(11)	0(0)	2(50)	0(0)	2(50)	3(100)	0(0)	0(0)	1(33)	0(0)	2(67)	1(100)	0(0)	0(0)
TZP	13(72)	2(11)	0(0)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	0(0)	1(33)			
CF	7(39)	4(22)	7(39)	3(75)	0(0)	1(25)	0(0)	1(33)	2(67)	1(33)	0(0)	2(67)	0(0)	0(0)	1(100)
CZ	12(67)	0(0)	6(33)	2(50)	0(0)	2(50)	1(33)	0(0)	2(67)	1(33)	0(0)	2(67)	0(0)	0(0)	1(100)
CXM	11(61)	0(0)	7(39)	3(75)	0(0)	1(25)	1(33)	0(0)	2(67)	1(33)	0(0)	2(67)	0(0)	0(0)	1(100)
CXMAX	12(67)	0(0)	6(33)	2(50)	0(0)	2(50)	0(0)	1(33)	2(67)	1(33)	0(0)	2(67)	0(0)	0(0)	1(100)
FOX	16(89)	1(6)	1(6)	2(50)	0(0)	2(50)	3(100)	0(0)	0(0)	1(33)	0(0)	2(67)	0(0)	0(0)	1(100)
CPD	13(72)	1(6)	3(17)	2(50)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	0(0)	1(33)	1(100)	0(0)	0(0)
CAZ	17(94)	0(0)	0(0)	1(25)	1(25)	2(50)	3(100)	0(0)	0(0)	2(67)			1(100)	0(0)	0(0)
CRO	16(89)	0(0)	2(11)	2(50)	1(25)	1(25)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	1(100)	0(0)	0(0)
FEP	16(89)	0(0)	2(11)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	1(33)	0(0)	1(100)	0(0)	0(0)
GM	16(89)	2(11)	0(0)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	0(0)	1(33)	1(100)	0(0)	0(0)
TM	15(83)	0(0)	3(17)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(670	0(0)	1(33)	1(100)	0(0)	0(0)
CIP	16(89)	0(0)	2(11)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	0(0)	1(33)	1(100)	0(0)	0(0)
LEV	16(89)	0(0)	2(11)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	2(67)	0(0)	1(33)	1(100)	0(0)	0(0)
TE	16(89)	0(0)	2(11)	2(50)	1(25)	1(25)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	1(100)	0(0)	0(0)
FT	12(67)	5(28)	1(6)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	1(100)	0(0)	0(0)
SXT	10(56)	0(0)	8(44)	3(75)	0(0)	1(25)	3(100)	0(0)	0(0)	3(100)	0(0)	0(0)	1(100)	0(0)	0(0)

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AMC=Amoxicillin/Clavulnic acid
TZP = Piperacillin/Tazobactam
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3. *If the sum of S, I and S is not equal to number of isolates (100%), it should be considered as AST test did not done for some of the species for that particular antimicrobia*

Table:4Antimicrobial Susceptibility Patterns Continued…….

Antimicrobials	Bacterial Isolates														
	A. hydrophila/cavae (No.=26)			*p. alkalificiens* (No.=10)			*P. retigerri* (No.=8)			*A. hydrophila* (No. =5)			*A. sobria* (No. =3)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AM	2(8)	0(0)	4(15)	1(10)	1(10)	8(80)	2(25)	0(0)	6(75)	0(0)	2(40)	3(60)	0(0)	0(0)	3(100
AMC	12(46)	10(38)	3(12)	1(10)	0(0)	2(20)	1 (13)	0(0)	2(25)	4(80)	1(20)	0(0)	2(67)	1(33)	0(0)
TZP	13(50)	2(8)	4(15)	6(60)	1(10)	0(0)	4(50)	0(0)	2(25)	4(80)	0(0)	1(20)	1(33)	0(0)	1(33)
CF	1(4)	2(8)	1(4)	2(20)	0(0)	6(60)	3(38)	1(13)	4(50)	2(40)					
CZ	12(46)	1(4)	10(38)	2(20)	0(0)	7(70)	3 (38)	0(0)	4(50)	3(60)	0(0)	2(40)	1(33)	0(0)	2(67)
CXM	17(65)	1(4)	7(27)	4(40)	1(10)	4(40)	5(63)	0(0)	3(38)	5(100)	0(0)	0(0)	1(33)	0(0)	2(67)
CXMA	17(65)	3(12)	6(23)	4(40)	0(0)	5(50)	4(50)	1(13)	3(38)	4(80)	1(20)	0(0)	1(33)	0(0)	2(67)
FOX	17(65)	0(0)	9(35)	6(60)	0(0)	2(20)	6(75)	0(0)	1(13)	4(80)	1(20)	0(0)	3(100)	0(0)	0(0)
CPD	18(69)	2(8)	5(19)	5(50)	2(20)	3(30)	4(50)	2(25)	2(25)	4(80)	1(20)	0(0)	1(33)	0(0)	2(67)
CAZ	24(92)	1(4)	1(4)	6(60)	1(10)	3(30)	4(50)	0(0)	4(50)	5(100)	0(0)	0(0)	2(67)	0(0)	1(33)
CRO	21(81)	1(4)	4(15)	6(60)	0(0)	4(40)	4(50)	2(25)	1(13)	5(100)	0(0)	0(0)	1(33)	1(33)	1(33)
FEP	4(15)	0(0)	1(4)	7(70)	0(0)	3(30)	7(88)	0(0)	1(13)	2(40)					
GM	23(88)	0(0)	3(12)	7(70)	0(00	2(20)	7(88)	0(0)	1(13)	4(80)	1(20)	0(0)	2(67)	1(33)	0(0)
TM	23(88)	2(8)	1(4)	9(90)	0(0)	1(10)	6(75)	0(0)	2(25)	4(80)	0(0)		1(33)	1(33)	1(33)
CIP	22(85)	2(8)	2(8)	8(80)	0(0)	2(20)	5(63)	0(0)	3(38)	4(80)	0(0)				
LEV	25(96)	0(0)	1(4)	8(80)	0(0)	2(20)	7(88)	0(0)	1(13)	4(80)	0(0)		3(100)	0(0)	0(0)
TE	21(81)	0(0)	5(19)	2(20)	0(0)	8(80)	1(13)	0(0)	7(88)	1(20)	0(0)	4(80)	3(100)	0(0)	0(0)
FT	25(96)	0(0)	1(4)	4(40)	0(0)	6(60)	3(38)	0(0)	5(63)	5(100)	0(0)	0(0)	3(100)	0(0)	0(0)
SXT	15(58)	0(0)	10(38)	6(60)	0(0)	4(40)	2(25)	1(0)	5(63)	4(80)	0(0)	1(20)	3(100)	0(0)	0(0)

Key *R=Resistant* *I= Intermediate* *Sensitive* *S= Sensitive*

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1. *IF S, I and R cells are Blank in the table, it shows that the machine did not test AST patterns of that particular antimicrobials for all number of bacteria mentioned on the same column.*
2. *If only S or I or R is written among the three options (S, I, R), it shows that only AST pattern of that particular antimicrobial is done for one of the isolates found on the same column.*
3. *If the sum of S, I and S is not equal to number of isolates (100%), it should be considered as AST test did not done for some of the species for that particular antimicrobial.*

5.3. Multidrug Resistance Patterns of the Total Isolates

Among identified bacterial species, most of them showed MDR property. *P .retigerri* showed 100%MDRfollowed by *P.alkalificiens* (90 %), *E.coli* (78 %), *Morganella* species (75%), and *C.frundi* (60%). Only 5% of the total isolates were 100 % sensitive to all tested antibiotics (table3).

Table 5: Multidrug Resistance Patterns for Tested Antibiotics against Isolates from river water June, 2017.

	Number of antimicrobials resisted No (%)									
Bacterial isolate	R0	R1	R2	R3	R4	R5	R6	R7	Total	MDR
A.hydrophila/ca (N=26)	2(8)	4(15)	10(38)	6(23)	1(4)	1(4)	2(8)	0(0)	26(100)	10(38)
E.coli (N=23)	1(4)	1(4)	3(13)	2(9)	3(13)	6(26)	6(26)	1(4)	23(100)	18(78)
K.pnumonia(N=18)	1(6)	7(39)	3(17)	2(11)	1(6)	3(17)	1(6)	0(0)	18(100)	7(39)
Raoutella species (N=18)	0(0)	6(33)	7(39)	3(17)	0(0)	1(6)	0(0)	1(6)	18(100)	5(28)
K.oxytoca(N=17)	0(0)	7(41)	3(18)	3(18)	2(12)	1(6)	0(0)	1(6)	17(100)	7(41)
C.frundi(N=10)	0(0)	2(20)	2(20)	1(10)	4(40)	1(10)	0(0)	0(0)	10(100)	6(60)
P.alkalificiense(N=10)	0(0)	1(10)	0(0)	2(20)	2(20)	5(50)	0(0)	0(0)	10(100)	9(90)
P. retigerrI(N=8)	0(0)	0(0)	0(0)	1(13)	4(50)	2(25)	0(0)	1(13)	8(100)	8(100)
A.hydrophila(N=5)	1(20)	1(20)	1(20)	2(40)	0(0)	0(0)	0(0)	0(0)	5(100)	2(40)
Morganella species (N=4)	0(0)	1(25)	0(0)	2(50)	1(25)	0(0)	0(0)	0(0)	4(100)	3(75)
E.clocae(N=3)	1(33)	0(0)	1(33)	0(0)	1(0)	0(0)	0(0)	0(0)	3(100)	1(33)
A.sobria(N=3)	0(0)	1(33)	1(33)	0(0)	1(33)	0(0)	0(0)	0(0)	3(100)	1(33)
Cryovcrescens(N=3)	1(33)	0(0)	1(33)	0(0)	1(33)	0(0)	0(0)	0(0)	3(100)	1(33)
E.gergovia(N=1)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
C.braki(N=1)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
Total	**8(5)**	**31(21)**	**33(22)**	**24(16)**	**21(14)**	**20(13)**	**9(6)**	**4(3)**	**150(100)**	**78(52)**

Key:

R0: No resistance for any class of antimicrobial R1: Resistance for one class of antimicrobials

R2: Resistance for two class of antimicrobial R3: Resistance for three class of antimicrobials

R4: Resistance for four class of antimicrobials R5: Resistance for five class of antimicrobials

R6: Resistance for six class of antimicrobials R7: Resistance for seven class of antimicrobials

6. Discussion

Bacteriological analysis of river water are used to assess its quality for human consumption, recreational purpose or agricultural activities to safe guard public health. Majority of the river water sources harboured enteropathogens and were also reported to be of poor microbiological quality and unsafe for consumption (6). The presence of enteric bacterial pathogens in water sources may spell health hazards such as diarrhoeal diseases, which accounts for a substantial degree of morbidity and mortality in adults and children (51). Management of diarrhoea may require the administration of antibiotics. However, several bacteria are known to be resistant to a wide array of antibiotics (38) The high rate of bacterial isolation from rivers preclude for direct domestic use and may also be problematic for flocculation and filtration purposes, with consequent increase in cost of water treatment. To my knowledge, this is the first study in Ethiopia, which has attempted to assess microbiological quality of river water, and antibiotic susceptibility of some bacterial isolates.

In this study, assessing the gram negative bacterial profile and antibiotic susceptibility patterns of river water sources were examined in order to establish physical safety of water sources and to provide updated data on antibiograms of enteric and non-enteric pathogens for better management of patients requiring empiric antibiotic therapy.

The isolation rate of gram negative bacteria from this study was 98% which is almost in agreement to other studies done in Romania, Spain, South Africa and Nigeria in which all (100%) of the samples were positive for one or more than one bacterial isolates (32, 38, 39, 40). Among 150 isolated gram negative bacterial species, *A. hydrophila/cavae* was the predominant bacteria accounted 26 (17%) followed by *E.coli* 23(15%), *K.pneumonia* 18(12%), *Raoultella* species 18(12%), *K. oxytoca* (11%) *C.freundi* 10 (7%) and p.alkalificience10 (7%) which is in agreement to other studies done in Romania and Mexico except *Providentia* and *Raoutella* species were not identified in the two studies (32, 35). However the study done in South Africa showed that *E.coli* was the predominant identified species which contradict with this study in which, *Aeromonas* species were the predominant bacterial isolates. This variation may be due to the fact that near to the study area there may be high number of health care institutions and level of discharged domestic and or healthcare liquid wastes to the receiving river may be very high.

The prevalence of *Raoutella* species, *K. pneumonia* and *K. oxytoca* was also high which accounted 18(12%), 18(12%), and 17(11%) respectively.This was similar to study done in Mexico except *Raoutella* species which is found only in this study (35). Not only in the study done in Mexico, *Raoutella* species not identified in many research areas. This may be due to the fact that *Raoutella* bacterial species identification with manual method which usually uses less than 30 biochemical tests may be very difficult unlike to this study which used BiomerieuxVitek 2 compact machine which identifies bacterial species by 64 biochemical tests. Geography is another factor that affects distribution of bacterial species; as the result *Raoultella*species may not found there (Mexico and Romania).

The predominant isolate, *A. Hydrophila/Cavae* 26(17%) which is higher than the study done in South africa (20 out of 201 (10%)) revealed high level of resistance to cefazolin 10 (38%), cefoxitin 9 (35%) and trimethoprim sulphomethoxazole 10 (38%). However, it showed very high sensitivity to levofloxacin, ceftazidime, tobramycin (gentamicin) and ciprofloxacin with the percentage of 96%, 92%, 89%, 85% respectively which is in agreement to the study done in South Africa in which more than 80% of isolated *Aeromonas* species were sensitive to gentamicin and ciprofloxacin (39). Average MDR rate of *Aeromonas* species was 39% which was higher than 3.4% reported in Spain (38). This variation may occur as the result of poor waste management practice in Addis Ababa, Ethiopia.

The second predominant Gram-negative isolate, *E.coli*. Showed also high level of resistance to many of tested antibiotics like ampicillin 21 (91%), cefalotin, cefuroxime, ceftriaxone and cefepime each accounted 16(70%). This was higher than results previously reported in Mexico (39.4%, 36.4%, 12.1%, 12.1% and 12.1%, respectively (35). However it was sensitive to nitrofurantoin 17 (74%) and tobramycin 14(61%). Even if it showed high sensitivity to those antimicrobials, level of sensitivity was lower than the sensitivity to tobramycin (100%) reported by the same study (35). This high resistance ratein this study may result from poor waste management practice, lack of treatment plants for domestic and health care institutions and poor antimicrobial usage in Addis Ababa. It showed 78% MDR rate which was very high compared to previously reported results in Romania (60.34%) (32) and Netherland (11%) (31).

Klebseilla species (*K.pneumonia*and*K.oxytoca*) were another bacterial strain which abundantly identified in this study accounted18 (12%) and 17(11%) respectively. Like other *Enterobactricaea*, both *K.pneumonia* and *K.oxytoca* showed high resistance to ampicillin

(94%) which was higher than 63.07% reported by Florica M. et al., 2015 (32). Resistance to penicillin antibiotics especiallyresistance to ampicillin becomes very common in the world(52) and results of this study also showed how much it increases.

However, both *K.pneumonia* and *K. oxytoca* species showed high sensitivity to levofloxacin 15(88%) and 15(83%) respectively. This result was almost comparable to 83.08% sensitivity reported by Florica M. et al., 2015 (32). Cefazolin and piperacillin/tazobactam are another antibiotics that are effective against *K.pneumonia*. High rate of MDRwas observedfor *Klebseilla*species and the average MDR rate was 40.5% which was higher than 32.76% which is reported by Florica M. et al., 2015 (32) and Marisol GU et al., 2000 (5.5%) (38). However, the current finding was lower than previously reported results in Mexico (50%) (35) and results reported in Brazil (77.5%) (37). This variation in different geographical area may be due to different antimicrobial usage practices.

Another enteric bacteria isolated was *C. frundi* and it accounted 10 (7%) isolation rate.*C.frundi* showed very high resistant to cefazolin, 8 (80.0%), amoxicillin/clavulnic acid 7 (70.0 %) and cefuroxime 7 (70.0 %). However, this result was lower than previously reported findings in Egypt; (100%) resistant to ampicillin and SXT (41). However, it showed high sensitivity to ceftazidime 8(80.0%), levofloxaxin 8(80.0%), gentamicin (70.0%), and tobramycin 7(70.0%).respectively. This was in agreement with reported results in Mexico in which more than 80 of the isolates were sensitive to many cephalosporin antibiotics (35) and Spain in which all isolates were 100 sensitive to aminoglycosides (gentamicin and tobramycin)(38).

Among three isolated *E.clocae*, 2(66.7%) of them showed high resistance to cefalotin, amoxicillin/clavulnic acid, cefazolin, cefuroxime, cefuroxime axetil and cefoxitin. However,all isolates were 100% sensitive to ceftriaxone, tetracycline, nitrofurantoin and trimethoprim-sulphomethoxazole. Multidrug resistance rates of e.clocae was 33% which was comparable to reports in Mexico (33%) (35) and Romania (34%) (38).

Another gram negative bacterial isolate, *P. retigerri* showed high resistant to tetracycline and amoxicillin/clavulnic acid with the percentage of 7(87.5%) and 6(75%) respectively. This result was lower than reported results in Egypt (100% resistant to ampicillin and tetracycline) (41). But 87.5% of the isolate showed high sensitivity to cefepime, gentamicin and levofloxacin. Another species identified with in the same genus *Providentia* was *P.alkalificiens* and it showed high resistant to ampicillin, tetracycline, cefazolin and

nitrofurantoin with the resistant rate of 8(80.0%),8 (80.0%),7(70.0%) and 6(60.0%) respectively. *P.retigerri* and *P. alkalificiens* showed multidrug resistant rate of 100% and 90% respectively.

About 18 *Raoutella* species was also identified and most of them were sensitive to ceftazidime17 (94.5%), cefoxitin 16(88.9%), ceftriaxone16 (88.9%), cefepime16 (88.9%), and amoxicillin/clavulnic acid 15(83.3%). Multidrug resistance rate of *Raoutella* species was very low (28%). *Morganella,Cryovcrysence*, and *A.sobria* species were bacterial species isolated in low number with multidrug resistant rates of 75% 33% and 33% respectively.

7. Strength and Limitation of the Study

7.1. Strength of the study

- ✓ Uses of VITEK 2 COMPACT system have a high potential to identify varies bacteria form sewage polluted river samples by using of 64 biochemical tests, which makes identification more simple and perform AST tests for 19 antimicrobials.
- ✓ VITEK 2 COMPACT system also minimizes personal error during species identification.

7.2 Limitation of the study

- ✓ It was not possible to include anaerobic bacteria due to lack of anaerobic laboratory setup
- ✓ Prevalence and AST pattern of Gram positive cocci was not determined due to facility constraint.
- ✓ Some bacterial isolates were not identified to the species level by the machine.
- ✓ Due to resource constraint enrichment media were not used for favourable growth of *salmonella* and shigella

8. Conclusion and recommendation

8.1. Conclusion

In this study, a total of 150 bacterial isolates were identified to the species level and the isolation rate was 98%. *Aeromonas* species and *E. coli* were the most abundant isolated bacteria. Most of antibiotics tested were resisted by isolated bacteria. Ampicillin was the least effective antimicrobials whereas aminoglycosides (gentamicin and tobramycin were the most effective antibiotics. Among flour quinolones, levofloxacin was the most effective. Very high MDR were observed. *P .rettgerii* showed the highest MDR to tested antibiotics Followed by *P.alkalificiens, E.coli* and *Morganella* species.

8.2 Recommendation

Antimicrobial and MDR become a big challenge to the world; so proper domestic and healthcare wastes management may be essential since it is the most probable cause for aquatic and terrestrial pollution.

Periodic review of antimicrobial susceptibility pattern of river water should be conducted through further more inclusive studies.

For researchers, it is better to undergo molecular characterization of antimicrobial resistance coding genes of the bacteria.

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10. Annexes

10.1. Annex I Laboratory Procedure

Bacteriological characterization of water

Specimen Collection

After wastewater is collected with sterile bottle it will be immediately transported to EPHI

Microbiology laboratory for further analysis.

Detection and isolation of pathogenic & potentially pathogenic bacteria

Specimen processing: I.

Culture

Inoculation of aerobic bacteria

Procedure:

1. Inoculate the specimen on 5%sheep blood agar and MacConkey agar.

2. Incubate in a humid environment at 37oc.

3. Incubate for a minimum of 48 hours before discarding the plates.

4. Examine the plates after 18-24 hours of incubation.

5. If there is growthsub culture to more selective media to get pure colonies. If ther is confusion, do gram stain.

Gram staining technique

1. Make smear from colonies grown on basic media

2. Fix the dried smear

3. Cover the fixed smear with crystal violet stain for 30–60 seconds.

4. Rapidly wash off the stain with clean water.

5. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.

6. Wash off the iodine with clean water.

7. Decolorize rapidly (few seconds) with acetone–alcohol.

8. Wash immediately with clean water.

Caution: Acetone-alcohol is highly flammable; therefore use it well away from an open flame.

9. Cover the smear with neutral red/sufranin stain for 2 minutes.

10. Wash off the stain with clean water.

11. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry.

12. Examine the smear microscopically, first with the 40X objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

MICROBIAL IDENTIFICATION USING THE BIOMÉRIEUX

VITEK® 2 COMPACT SYSTEM

PRINCIPLES

The VITEK 2 Compact is semi-automated microbiology system utilizing growth-based technology. This format focuses on the industrial microbiology-testing environment while also having application for low to middle volume clinical laboratories. The colorimetric reagent cards (GN, GP, and YST) apply to all system formats for both industrial and clinical laboratories.

Reagent Cards

The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vesselthat prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation. Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system.

There are currently four reagent cards available for the identification of different organism classes as follows:

1. GN - Gram-negative fermenting and non-fermenting bacilli

2. GP - Gram-positive cocci and non-spore-forming bacilli

3. YST - yeasts and yeast-like organisms

4. BCL - Gram-positive spore-forming bacilli

Culture Requirements

The on-line product information contains a culture requirements table that lists parameters for appropriate culture and inoculum preparation. These parameters include acceptable culture media, culture age, incubation conditions, and inoculum turbidity.

Suspension Preparation

A sterile swab or applicator stick is used to transfer a sufficient number of colonies of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube. The turbidity is adjusted to 0.5-0.63 Mac Farland standard and measured using a turbidity meter called the DensiChekTM.

Inoculation

Identification cards are inoculated with microorganism suspensions using an integrated vacuum apparatus. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube. The cassette can accommodate up to 10 tests. The filled cassette is placed either manually into a vacuum chamber station. After the vacuum is applied and air is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

Card Sealing and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. The carousel incubator can accommodate up to 30 or up to 60 cards. All card types areincubated on-line at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period.

Optical System

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15

minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present.

Test Reactions

Calculations are performed on raw data and compared to thresholds to determine reactions for each test. On the VITEK 2 Compact, test reaction results appear as "+"," –", "(–)" or "(+)". Reactions that appear in parentheses are indicative of weak reactions that are too close to the test threshold.

Database Development

The databases of the VITEK 2 identification products are constructed with large strain sets of well-characterized microorganisms tested under various culture conditions. These strains are derived from a variety of clinical and industrial sources as well as from public (e.g., ATCC) and university culture collections.

Analytical Techniques

Test data from an unknown organism are compared to the respective database to determine a quantitative value for proximity to each of the database taxa. Each of the composite values is compared to the others to determine if the data are sufficiently unique or close to one or more of the other database taxa. If a unique identification pattern is not recognized, a list of possible organisms is given, or the strain is determined to be outside the scope of the database.

Identification Levels

An unknown bio-pattern is compared to the database of reactions for each taxon, and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation.

Mixed Taxa Identifications

This occurs when the bio-pattern is representative of a collective taxon and generates a genus-level, group-level, or slashline identification. In rare cases, species-level identification can be a mixed taxon comprised of two subspecies.

Supplemental tests may be used to delineate representative species or subspecies of these collective taxa.

Supplemental Testing

In the case of low discrimination identifications, two or three choices are listed in the order of their probability calculations. However, all taxa appearing in a low discrimination identification are viable choices and should only be ruled out after additional testing and/or observation. The lab report contains recommended supplemental tests that allow for differentiation of choices in low discrimination identifications. If the supplemental tests listed are insufficient to complete the identification, then standard microbiology references should be consulted.

Non-Reactive Biopattern

When a biopattern is calculated for an unknown organism that is either completely negative or consists of both negative tests and tests with reactions that lie too close to the test thresholds, the identification result will be "Nonreactive biopattern." If one encounters a non-reactive biopattern, a note will appear that states: "Organism with low reactivity biopattern – please check viability." The species that can potentially trigger this note are shown in separate tables for each of the respective products described below.

APPLICATIONS

GN Card

The GN card is used for the automated identification of 135 taxa of the most significant fermenting and non-fermenting Gram-negative bacilli. The list of claimed species is shown in Table 3. Of the 135 taxa, 12 are grouped taxa in either genus (3), group (6), or slashline (3) designations. When representative taxa of these collective designations are included, the total number of taxa claimed by GN is over 160. As with low discrimination identifications, grouped taxa can be separated into their component taxa by supplemental testing and/or observation.

The GN card is based on established biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance (ASM 1998, Atlas 1993, Brenner et al. 1993, Chang et al. 2002, Coenye et al. 2001a, Coenye et al. 2001b, De Baere et al. 2001, Freney et al. 2000,Gavini et al. 1989, Holt et al. 1994, Krieg and Holt

1984, Murray et al. 1999, Richard and Kiredjian 1992, Smith et al. 1991, Vandamme et al. 1999). There are 47 biochemical tests and one negative control well. Final identification results are available in approximately 10 hours or less. The list of test substrates is shown in Table 7. In a recent multi-site study, the performance of the VITEK 2 GN was evaluated using 562 isolates of both commonly and rarely observed species ofwww.pda.org/bookstore Microbial Identification Using the bioMérieux VITEK® . . . 9 Gram-negative bacilli, including 153 non-fermentative strains. The reference identification was determined with api® 20 E and api 20 NE identification kits.

Overall, the VITEK 2 GN correctly identified 96.8% of the isolates, including

6.4% low discrimination with the correct species listed. Misidentifications occurred at 3.0% and no identifications occurred at 0.2%.

10.2. Annex II: sample collection form

Water sample collection form

1. Name of sample collector_____
2. Sub city_____
3. Sample ID_____
4. Sampling site_____
5. Source_____
6. Date of collection_____/_____/_____
7. Time of collection_____
8. Date of processing_____
9. Time of processing_____

10.3. Annex III: Declaration

I, the undersigned, Diagnostic and Public Health Microbiology MSc. student declare that this thesis paper is my original work for fulfilment of the requirements for degree of Master of Science in Diagnostic and Public Health Microbiology.

Name: TeshomeBelachew

Signature: _____

Place of submission: Department of Diagnostic and Public Health Microbiology, School of Allied Health Science, College of Medicine and Health Science, Addis Ababa University

Date of Submission: _____

This thesis paper is submitted for confirmation with my / our approval as University advisor (s)

Advisor

Mr. KassuDesta (Assistant Professor): Sign: _____ Date: _____