

Antibiotic Resistance Profiles among Enteric Bacteria Isolated from Wastewater in Septic Tanks

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Abstract

This study was carried out to assess the antibiotic resistance profiles of 95 isolates of enteric bacteria obtained from wastewater in septic tanks within Chuka University in Kenya which houses laboratories, animal farm, crop demonstration and research farm, student hostels and a health Centre. The isolates were differentiated into four genera. *E.coli* was the predominant organism (41 isolates). The others were *Salmonella* (19), *Proteus* (13) and *Klebsiella* (22). The strains were subjected to different classes of antibiotics including β -lactams, macrolide, tetracycline and sulfa drugs. The study revealed an increase in resistance to Penicillins and a decreased resistance to the tetracycline and the cephalosporin assayed. 84.2% (80) of the strains resisted amoxyclav, 76.8% (73) ampicillin and 64.2% (61) oxacillin. Others were vancomycin 47.4% (45), erythromycin 47.4% (45), co-trimoxazole 41.1% (39), doxycycline 36.8% (35) and ceftazidime 31.6% (30). All the strains recorded multiple drug resistance with *E.coli* being the most resistant to the antibiotics, followed by *Salmonella*, *Klebsiella* and *Proteus*. Drug resistance in *E.coli* was significantly different from the other strains, $P < 0.05$. However, drug resistance between *Salmonella* and *Klebsiella* strains was not significantly different ($P > 0.05$). Drug sensitivity among the four bacterial genera was not significantly different ($P = 0.01$). It is anticipated that the findings of this study will provide an understanding of the changing antibiotic resistance trends in enteric bacteria from sewage effluent and help design improved sewage treatment strategies to contain the spread of drug resistance which poses a great public health risk. The report also emphasizes the necessity of continuous surveillance of antibiograms of enteric bacteria in order to rationalize their treatment protocols.

Keywords: Enteric bacteria; multidrug resistance; septic tanks; wastewater.

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1. Introduction

Sewage water in a septic tank results from the discharge into the sewers of human excreta and wastewater from a community. It has high content of both organic and inorganic matter, as well as high densities of living organisms, including pathogenic, commensal and environmental bacteria. This characteristic makes sewage a particularly suitable niche for spread of antibiotic resistance [12]. Sometimes the acquisition of antibiotic resistance is not dependent on the presence of antibiotics but a multitude of other substances occurring in sewage such as heavy metals and biocides. Bacterial resistance to antimicrobial drugs has emerged as a global concern [31]. Fuelling this concern is the widespread detection of multidrug resistant enteric pathogens. Antibiotics are extensively used in human, veterinary and agriculture and considered as the most important factor promoting the emergence, selection and dissemination of resistant organisms [18].

Urban areas constantly exposed to the large variety of antibiotics that are commonly used in the hospital and the communities have considerable reservoirs of resistance. Thousands of people receive antibiotic treatment every day in hospitals, in nursing homes and at home and accordingly many antibiotics are released as part of waste stream of the sanitary sewer system into wastewater. Septic tanks store wastewater and are hotspots with significant concentrations of antibiotic resistant microbes containing multiple resistance genes from a wide variety of antibiotics and other antimicrobial agents [12]. Septic tanks and other wastewater treatment facilities provide ideal environments for gene exchange and gene acquisition has been confirmed by studies of antibiotic resistance plasmids isolated from wastewater treatment plant bacterial cultures [32]. Sequencing of purified plasmid DNAs has demonstrated that very complex genomes are formed as evidenced by the combinations of resistance genes, transfer factors, transposases, intergrons and their associated integrases, thus a considerable level of genetic mixing and matching occurs with the consequence that novel combinations of antibiotic resistance determinants may be present in wastewater effluents [28].

The use of disinfectants, sterilants, Chemical pesticides, heavy metals, household products along with antibiotics creates a selective pressure in the environment that leads to the mutations in microorganisms that allow them to survive and multiply [2]. The present study was conducted to assess the changing patterns in antibiotic susceptibilities among enteric bacteria isolated from wastewater in septic tanks that serve as sewage reservoirs in the university with a view to provide a comprehensive understanding of the current trends in drug resistance among enteric pathogenic gram negative bacteria.

2. Materials and Methods

2.1. Sampling

Wastewater samples were purposively drawn from two separate septic tanks using sterile Teflon bottles in the month of June and July 2016. The samples were transported to the laboratory and assayed for viable enteric bacteria on selective and differential media.

2.2. Isolation of bacteria

The sewage effluent samples were serially diluted and 0.1 ml from the last two diluents (10^4 and 10^5) plated in both MacConkey agar and Eosin Methylene blue (EMB) agar plates to obtain enteric bacteria and coliform colonies. All the plates were incubated for 24 hours at 35°C . Distinct and representative colonies from both MacConkey and Eosin methylene blue agar plates were further purified and stored in nutrient agar slants in the refrigerator.

2.3. Bacterial characterization

Initial characterization was done based on the cultural characteristics and colony morphology. The strains were differentiated into *E.coli*, *Salmonella*, *Proteus* and *Klebsiella*. Green metallic sheen colonies on Eosin methylene blue agar plates were presumptive characteristics of *E.coli* which also appeared pinkish on MacConkey agar. Non-lactose fermenting colonies of *Salmonella* and *Proteus* were distinguished from lactose fermenting *Klebsiella* and *E.coli* colonies on MacConkey agar. The strains were further subjected to biochemical tests and confirmed based on criteria of Cheesebrough [8], Cappuccino and Sherma [6] and Harley and Prescott [14]. The tests included Gram-staining, production of H_2S , starch hydrolysis, production of acids and gas from carbohydrates, oxidase test, motility test, citrate utilization, Indole, methyl red and voges-proskauer test.

2.4. Determination of antibiotic susceptibility

The bacterial strains were inoculated into Mueller Hinton broth and incubated for 24 hours at 35°C . Using the disc diffusion method of Bauer and his colleagues [3] on Mueller Hinton agar plates, the strains were assayed for their sensitivities to the following: Ampicillin (10mcg), Oxacillin (1mcg), Co-trimoxazole (25mcg), Ceftazidime (30mcg), Doxycycline (30mcg), Erythromycin (15mcg), Vancomycin (30mcg) and Amoxyclav (20/10mcg). The antibiotic discs were obtained from Himedia laboratories Pvt. Ltd, Mumbai India. The agar plates were incubated for 18 to 24 hours at 35°C . The diameters of the zones of inhibition were measured to the nearest millimeter for each of the antibiotics tested. The antibiotic breakpoints were interpreted using standard recommendations of the National Committee for Clinical Laboratory Standards (NCCLS), [25]. Control plates were incubated without the antibiotic discs.

2.5. Limitation

The bacterial strains were not characterized to the species level or their serotypes due to lack of molecular technology and immunological facilities.

3. Results

A total of ninety five strains of enteric bacteria were obtained and differentiated into four genera. *E.coli* (41), *Salmonella* (19), *Proteus* (13) and *Klebsiella* (22). All the strains were subjected to the eight antibiotics impregnated in discs and their resistance profiles recorded. Amoxyclav had the highest incidence among the strains (84.2%), followed by ampicillin (76.8%) and oxacillin (64.2%). The strains recorded least resistance to ceftazidime (31.6%) and doxycycline (36.8%). The rest of the antibiotics vancomycin, erythromycin and cotrimoxazole recorded a slightly below average percentage resistance among the strains (47.4%, 47.4% and

41.2%) (Fig.1).

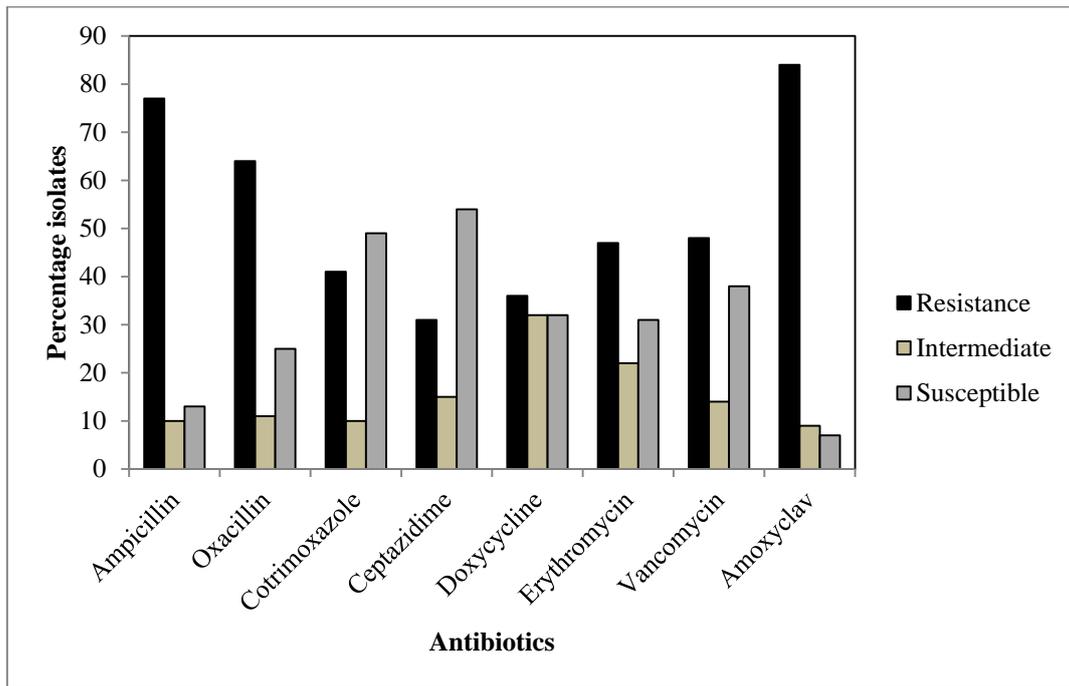


Figure 1: Percentage drug resistance among all the 95 bacterial strains

Each of the four bacteria genera recorded multiple drug resistance with each recording resistance to all the antibiotics at varying degree. Resistance among *E.coli* strains was significantly different from the other strains ($p < 0.05$). The order of resistance emerged as follows-; *E.coli*, *Salmonella*, *Klebsiella* and *Proteus*. It was however observed that drug resistance between *Salmonella* and *Klebsiella* isolates was not significantly different ($P > 0.05$) (Fig. 2).

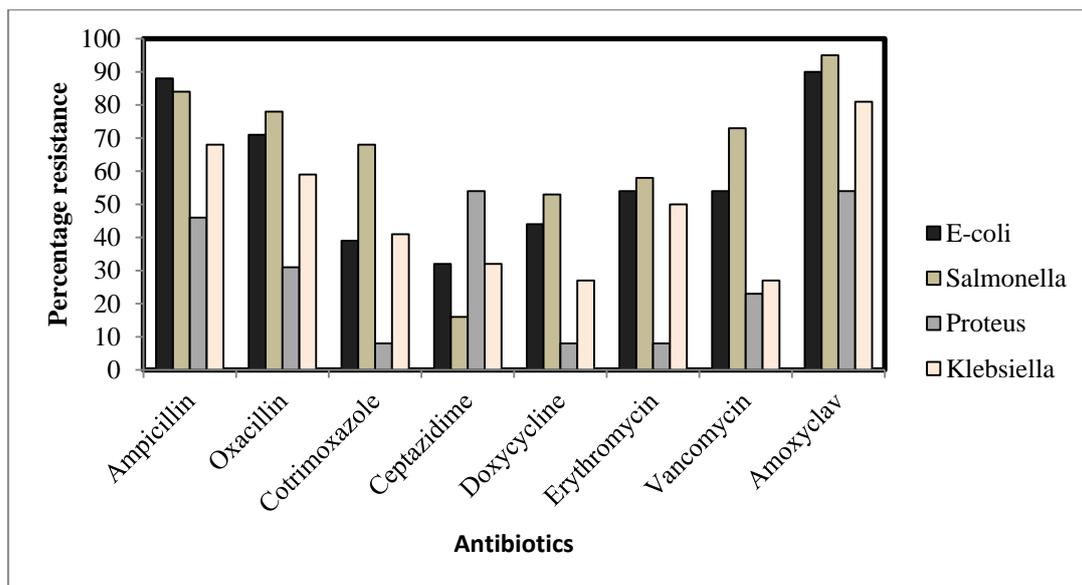


Figure 2: Percentage multidrug resistance among the four bacterial genera

E.coli, *Salmonella* and *Klebsiella* were most resistant to amoxyclav 90.2%, 95.7% and 81.8%, ampicillin 87.8%, 84.2%, 68.2% and oxacillin 70.7%, 78.9% and 59.1%. These isolates were least resistant to ceftazidime 31.7%, 15.7% and 31.8%. The rest of the antibiotics recorded relatively average resistance among these isolates. *Proteus* had notable resistance to ceftazidime in addition to amoxyclav and ampicillin (Table 1).

Table 1: Percentage isolates resistance to antibiotics

Antibiotic	% resistance			
	<i>E.coli</i> spp	<i>Salmonella</i> spp	<i>Proteus</i> spp	<i>Klebsiella</i> spp
Ampicillin	87.8	84.2	46.2	68.2
Oxacillin	70.7	78.9	30.8	59.1
Co-trimoxazole	39	68.4	7.7	40.9
Ceftazidime	31.7	15.7	53.8	31.8
Doxycycline	43.9	52.6	7.7	27.3
Erythromycin	53.6	57.8	7.7	50.0
Vancomycin	53.6	73.7	23.1	27.3
Amoxyclav	90.2	95.7	53.8	81.8

Although ceftazidime recorded high sensitivity among *E.coli*, *Salmonella* and *Klebsiella* Isolates, *Proteus* isolates were highly sensitive to erythromycin, doxycycline and co-trimoxazole. The order of sensitivity among all antibiotics was as follows; *Proteus*, *Salmonella*, *Klebsiella* and *E.coli* (Table 2). However, the drug sensitivity was not significantly different among the four genera (P= 0.10).

Table 2: Percentage isolates sensitivity to antibiotics

Antibiotic	% sensitivity			
	<i>E.coli</i> spp	<i>Salmonella</i> spp	<i>Proteus</i> spp	<i>Klebsiella</i> spp
Ampicillin	7.3	5.3	30.8	18.2
Oxacillin	24.4	10.5	38.5	31.8
Co-trimoxazole	43.9	31.6	76.9	59.1
Ceftazidime	53.7	68.4	30.8	54.5
Doxycycline	12.2	26.3	84.6	41.0
Erythromycin	17.1	10.5	84.6	41.0
Vancomycin	26.8	15.8	76.9	54.5
Amoxyclav	4.9	0	30.8	4.5

4. Discussion

Indiscriminate use of antibiotics has led to an increasing incidence of antibiotic resistance among strains of enterobacteriaceae worldwide [11, 17]. The findings of this study revealed that sewage effluent in the septic

tanks from the laboratories, health Centre, hostels and the crop and animal farms is heavily loaded with multiple drug resistant enteric bacteria and can be a plausible source of contamination to other natural water bodies. Antimicrobial drug resistance is a growing problem among organisms that cause diarrhea and other diseases. The acquisition of antimicrobial resistance provides a mechanism for survival of a bacterium in an otherwise hostile environment. The resistance is generally plasmid encoded or transposons mediated [22, 7]. The plasmids can move between species and even genera, accumulating genes by conjugation or recombination.

All the strains recorded resistance to all the antibiotics tested. Such multiple antibiotic resistances are known to arise due to acquisition of resistance genes through genetic exchange, mutation and physiological mechanisms such as possession of specific proteins and efflux pumps [15, 16]. Transfer of R-factors to pathogenic members of enterobacteriaceae especially *E.coli* and *Salmonella* is of grave concern particularly in controlling typhoid, diarrhea and other gastro-intestinal infections in humans [1, 30].

Multidrug resistance in *E.coli* and *Salmonella* strains in this study is particularly significant because they are associated with gastrointestinal illnesses acquired through contaminated water and food. Recent data from Gabon, Kenya, Nigeria, Senegal and Tanzania suggest that resistance among causative organisms of diarrhea infections such as enterotoxigenic, enteropathogenic and enteroaggregative *Escherichia coli* is high and appears to be rising [33, 26]. Notable drug resistant enteropathogenic *E.coli* outbreaks and sporadic cases have been reported from several African countries including Kenya and Tanzania [33, 29]. The finding of the more recently defined enteroaggregative *E.coli* which was found to be typically multidrug resistant and one of the most common causes of childhood diarrhea [34] is in line with the reported multidrug resistance among *E.coli* strains in this study.

The emergence and spread of multidrug resistant *Salmonella enterica* subsp. *Typhi* worldwide has had important consequences for mortality rates from typhoid fever [10]. Multidrug resistant nontyphoidal *Salmonella spp* (NTS) have emerged as a global public health threat. Studies from Kenya have found that community acquired NTS are among the top three causes of death among children <5 years of age [20, 4]. Multidrug resistance among *Salmonella* strains evident in this study correlates with other reports. Other studies have reported multidrug resistance in *Klebsiella* and *Proteus spp.* which are medically significant among the causative agents of urinary tract infections common among all the age groups [11].

Generally the study recorded high incidence of resistance among the enteric bacteria to the β -lactams amoxyclav, ampicillin and oxacillin. High resistance of β -lactams among enteric bacteria from the environment has been reported worldwide [19]. Increased β -lactam resistance among clinical isolates of *Pseudomonas* and *Staphylococcus* is a common problem. However high incidence against enteric bacteria is less frequently reported [27]. Similar high incidence of resistance to ampicillin and oxacillin among enteric bacteria was reported by Mohammad and his colleagues [23]. They also reported high sensitivity to cephalosporins such as cefuroxime and cefotaxime as well as protein inhibiting antibiotics like doxycycline and tetracycline. A similar observation was made in this investigation with the highest sensitivity recorded by the cephalosporin-ceftazidime among most of the strains and doxycycline among *Proteus* strains. The high sensitivity to doxycycline is an indication that tetracyclines are not widely used in the treatment of the animal infections in the

farm and may be less abundant in the wastewater. Therefore the enteric bacteria have not selected for their resistance. Other researchers have reported increased resistance to tetracyclines due to their continued use as growth promoters in swine and cattle [13]. Their resistance became apparent in 1970s when it was widely reported among Enterobacteriaceae, *Staphylococci*, *Streptococci* and *Bacteroides* spp [21]. Their resistance determinants are now widespread among both gram negative and gram positive bacteria [9]. Vancomycin, commonly used against gram positive bacteria is not highly active against gram negative bacteria because their outer membrane is impermeable to large glycopeptide molecules. This may be attributed to the observed resistance to this antibiotic. Resistance to the sulfa drug cotrimoxazole observed in this study is supported by studies done by Monchy and his colleagues [24] and Boga and his colleagues [5] on gram negative bacteria.

5. Conclusion

Resistance among the β -lactams amoxycylav, ampicillin and oxacillin is on the rise and on the decrease in the cephalosporin- ceftazidime. The resistance profiles observed among the different strains may be attributed to excessive use of different antimicrobial agents such as antibiotics in the treatment of bacterial infections in farm animals and the human population, use of chemical pesticides on crops, use of disinfectants such as phenolic compounds in cleaning washrooms, as well as the discharge of toxic metals from the laboratories into the sewer system. Bacterial exposure to these antimicrobial agents has contributed to selection of multidrug resistant strains in the environment. Although antibiotics are indispensable in the treatment of infectious and other diseases, the widespread development of antibiotic resistance in the community makes it imperative that the search for new novel bioactive compounds be maintained.

6. Recommendations

The findings of this investigation highlight the importance of safe disposal of sewage effluent within the institution and an urgent need to establish a wastewater treatment infrastructure to serve the university community along with the adjacent Chuka town population to contain the spread of drug resistant pathogenic bacteria in the environment as the use of septic tanks which is the general practice within the area poses a great public health risk emanating from possible contamination of water bodies through surface runoff during heavy rains. A concerted effort is necessary for the government, the pharmaceutical industry and the academia to cooperate in actions that will curtail resistance development in pathogens and support efforts to provide a continuing supply of potent antimicrobial agents.

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