

Multiple-Antibiotic Resistance Pattern of Coliform Bacteria Isolated from Different Sources in Iwo, Nigeria

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Abstract

Antibiotic resistance has become a big public health concern. Experiments were carried out to assess the antibiotic resistance pattern in coliform bacteria isolated from different sources in Iwo, Nigeria. Coliform bacteria were isolated on nutrient and xylose lysine deoxycholate (XLD) agar media and identified using standard procedures. Antibiotic multidisc containing ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), augmentin (30µg), nitrofurantoin (300µg), and ampicillin (10µg) were used to study the resistance patterns of the isolates. Statistical analyses were carried out using the analysis of variance test (ANOVA) and the PAST (paleontology statistics) software analytical package where applicable. Twenty-five (25) Coliform bacteria consisting of four genera were isolated and identified. *Enterobacter* had the highest occurrence (44%) followed by *Klebsiella* (24%), *E. coli* (20%) while *Citrobacter* had the lowest occurrence (12%). Overall more Coliform isolates were recovered at the abattoir (8), cafeteria (6), poultry (5), cow ranch (4) and hostel sewage (2). The highest resistance observed by the isolates was to ampicillin (91%) followed by augmentin (55%), while many of the isolates were sensitive to ceftazidime, gentamicin, ofloxacin and ciprofloxacin. Polymerase chain reaction (PCR for 16S RNA) method was carried out to screen for CTX-M gene in beta-lactam resistant isolates. A number of organisms have developed multiple resistances to several antibiotics and of major concern are Coliforms in which new resistance characteristics have been discovered leading to increasing resistance to drug therapies. This may become a great public health concern if not curbed.

Keywords

Coliforms, Antibiotic Resistance, CTX-M Gene, β-lactam Resistance, 16S RNA

1. Introduction

During the last few decades, the incidence of microbial infections has increased dramatically [8]. Continuous deployment of antimicrobial drugs in treating infections has led to the emergence of resistance among the various strains of microorganisms. Multiple-antibiotic resistance (MAR) is defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it [26]. According to WHO, these

resistant microorganisms are able to combat attack by antimicrobial drugs, which leads to ineffective treatment resulting in persistence and spreading of infections. Although the development of MAR is a natural phenomenon, extensive rise in the number of immune-compromised conditions, like HIV-infection, diabetic patients, individuals who have undergone organ transplantation, and severe burn patients, makes the body an easy target for hospital-acquired infectious diseases, thereby contributing to further spread of MAR [24].

Multiple-antibiotic resistance occurs when bacteria are resistant to more than one antibiotic. Because of years of

antibiotic overuse, it is now the rule rather than the exception among resistant bacteria [3]. This situation has largely occurred through the sequential use of different multiple antibiotics. The evolution of antibiotic resistance has been described as the most important evolutionary change in modern time, causing prolonged illness and increased cost of hospitalization for diseases that were once straightforwardly controlled [7]. Coliform bacteria are generally regarded as harmless indicators however, these bacteria may carry extra-chromosomal genetic elements determining resistance to antibiotics (R factors) which are transmissible between members of the Enterobacteriaceae as well as to other pathogenic and non-pathogenic bacteria [21]. The purpose of this study was to investigate the incidence of multiple antibiotic resistance among coliform bacteria from different sources in Iwo, Nigeria.

2. Materials and Method

Samples used for this research were collected from different sites in Iwo, Nigeria. The sites include: abattoir, poultry, cafeterias, hostel sewage, and cow ranch. Three different agar media including nutrient agar medium, peptone water medium, xylose lysine deoxycholate (XLD) agar medium were prepared according to the manufacturers specifications which were used for serial dilutions and streaking procedures for isolating the bacterial isolates. Coliform bacteria were isolated and identified using standard morphological and biochemical tests. Antimicrobial susceptibility tests were performed where the minimum inhibitory concentrations (MIC) were determined using a standard agar dilution method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute's (CLSI) guidelines using Gram negative antibiotic multidiscs containing eight (8) antibiotics which are; ceftazidime (30 μ g), cefuroxime (30 μ g), gentamicin (10 μ g),

ciprofloxacin (5 μ g), ofloxacin (5 μ g), amoxycilin (30 μ g), nitrofurantoin (300 μ g), and ampicillin (10 μ g). CTX-M β -lactam resistance genes were isolated (using PCR for 16S RNA method) and separated by gel electrophoresis.

3. Results

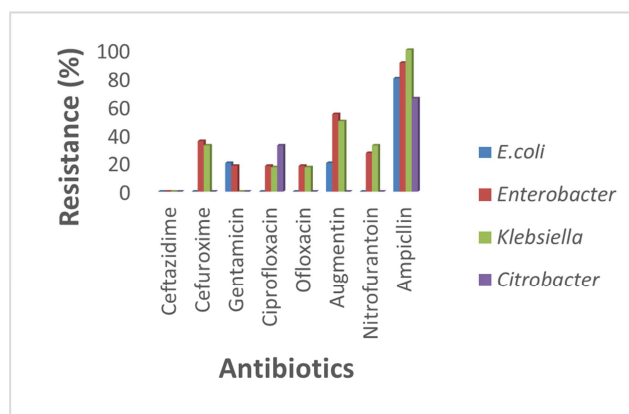


Figure 1. Resistance profile of Isolated Coliform bacteria to tested antibiotics.

The highest antibiotic resistance observed by the isolates was to ampicillin (all the coliform bacteria were highly resistant to ampicillin at $\geq 68\%$)

The prevalence of Coliform bacteria from different sources in Iwo, Nigeria was studied, four genera were identified (*E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*) and subjected to antibiotic sensitivity testing procedures. Figure 1 shows the resistance profile of the isolated Coliform bacteria to the tested antibiotics. The isolates (belonging to the four genera) were completely susceptible to ceftazidime but highly resistant to ampicillin at $\geq 68\%$)

Table 1. Antibiotic sensitivity of isolates resistant to three or more tested antibiotics.

Organism	Antibiotics (zones of inhibition in mm)							
	Ceftazidime	Cefuroxime	Gentamicin	Ciprofloxacin	Ofloxacin	Augmentin	Nitrofurantoin	Ampicillin
<i>Enterobacter</i> sp	29.5 \pm 0.5*	-	21.5 \pm 1.5	29.0 \pm 1.0	26.0 \pm 1.0	-	-	-
<i>Klebsiella</i> sp	32.0	-	20.5 \pm 2.5	30.5 \pm 0.5	25.0	-	-	-
<i>Klebsiella</i> sp	30.0	-	20.5 \pm 0.5	29.5 \pm 0.5	25.0	-	-	-
<i>Enterobacter</i> sp	29	17.0 \pm 1.0	-	-	-	-	19.5 \pm 0.5	-
<i>Enterobacter</i> sp	26.5 \pm 0.5	-	13.5 \pm 0.5	30.0	27.0 \pm 1.0	-	-	-
<i>Enterobacter</i> sp	24.0 \pm 1.0	-	19.5 \pm 0.5	26.0	26.0 \pm 1.0	-	-	-
<i>Enterobacter</i> sp	27.0 \pm 1.0	20.5 \pm 0.5	-	-	-	4.0 \pm 4.0	26.0 \pm 1.0	-
<i>Klebsiella</i> sp	28.5 \pm 0.5	15.0	12.5 \pm 0.5	-	-	5.0	20.0 \pm 2.0	-
<i>E. coli</i>	26.5 \pm 1.5	20.0	-	25.0 \pm 1.0	22.0 \pm 2.0	-	25.5 \pm 0.5	-
<i>Enterobacter</i> sp	11.0 \pm 1.0	-	9.0 \pm 1.0	21.0	20.5 \pm 0.5	-	16.5 \pm 0.5	-

* = Standard error of the mean.

From this study ten of the coliform isolates were resistant to three or more of the tested antibiotics. These isolates were highly resistant to cefuroxime, augmentin and ampicillin (100% resistance)

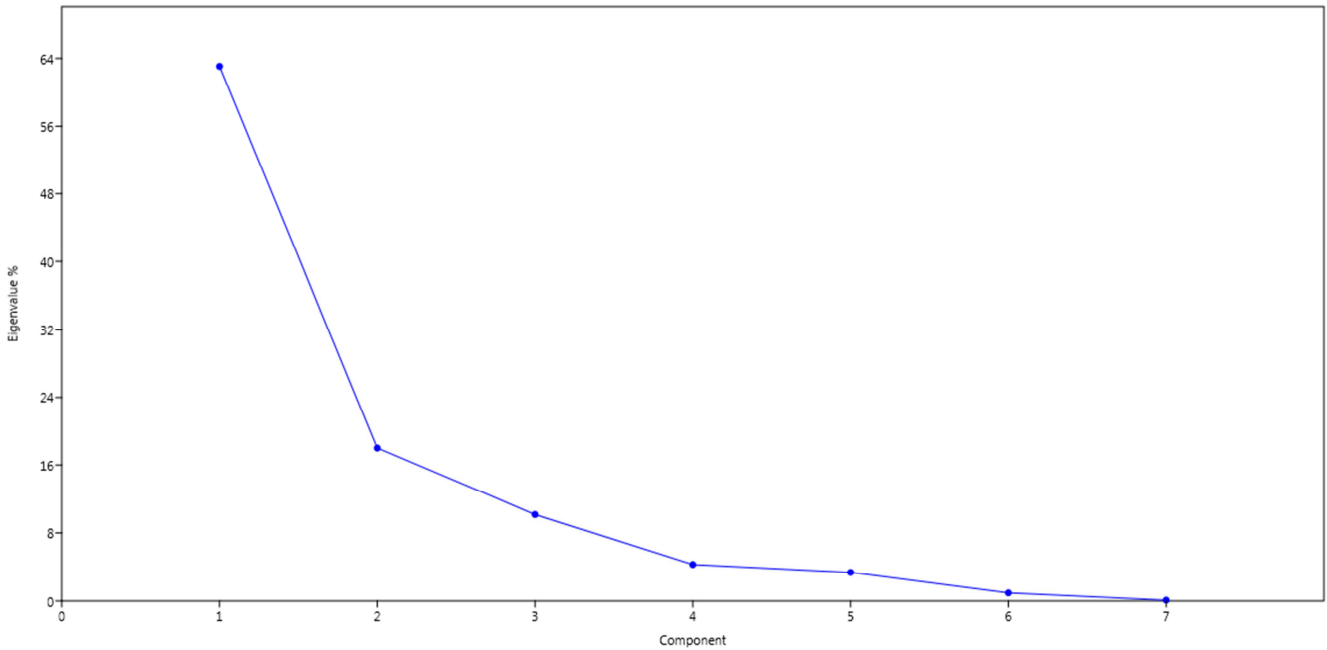


Figure 2. Scree plots of isolates resistant to three or more antibiotics.

Key: 1-Ceftazidime, 2- Ciprofloxacin, 3-Ofloxacin, 4-Gentamicin, 5- Nitrofurantoin, 6-Cefuroxime, 7-Augmentin.

The scree plot (figure 2) shows the degree of susceptibility of the multiple resistant isolates to the tested antibiotics. Starting from '1' (ceftazidime) with the highest percentage susceptibility to '7' (augmentin) with the lowest percentage susceptibility. This excludes ampicillin with 100% resistance.

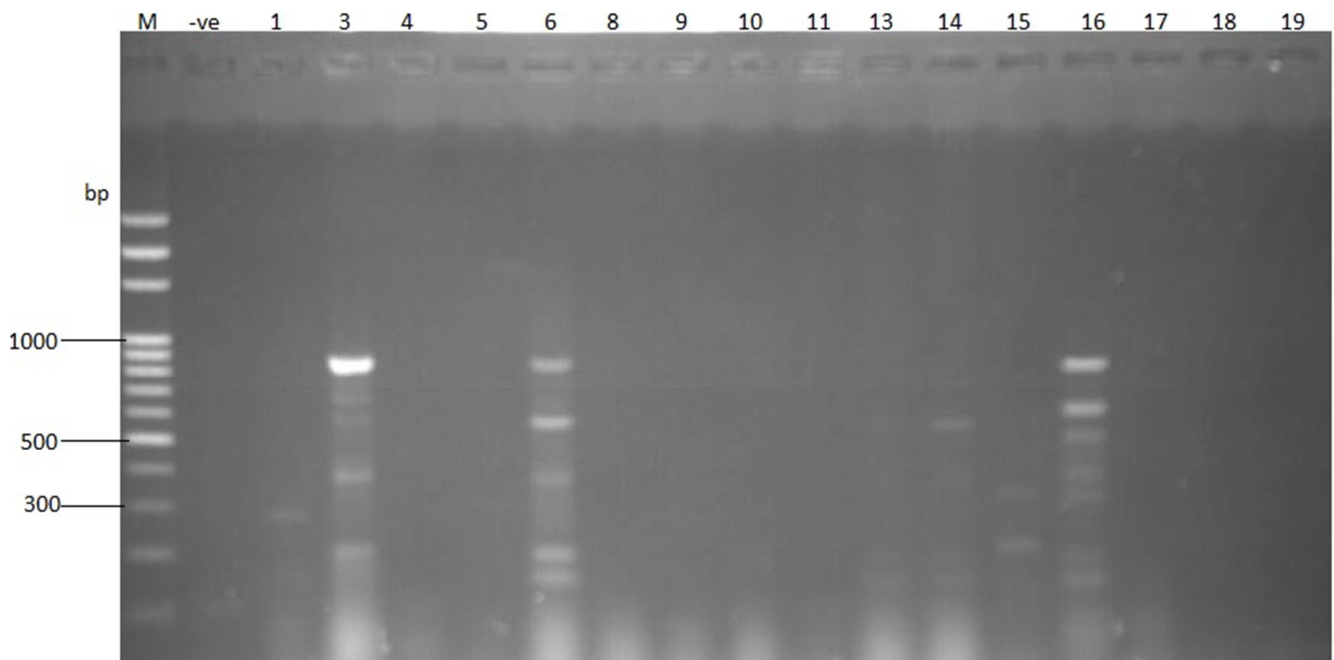


Figure 3. Agarose gel electrophoresis of PCR- amplified CTX-M genes (at 60°C annealing temperature) from beta-lactamase resistant Coliform isolates (isolates resistant to three or more antibiotics).

Lane 1: Maker, Lane 2: negative control, Lane 4: *Enterobacter* sp (3), Lane 7: *Klebsiella* sp (6), Lane 15: *Enterobacter* sp (16).

4. Discussion

The emergence of multiple antibiotic resistance is clearly related to the quantity of antibiotics and how they are being used [17]. Multiple antibiotic resistance may reflect acquisition of different resistance determinants on the same DNA molecule or single determinants, such as multiple antibiotic pumps that specify efflux activity against different antibacterials [17] & [22].

Four different Coliform bacteria isolated from different sampling sites during this study were subjected to different antibiotics. Many of the isolates exhibited high resistance to ampicillin and low resistance to three of the antibiotics (gentamicin, ciprofloxacin and ofloxacin). From the study *Enterobacter* isolates exhibited high level of resistance to augmentin and the highest percentage resistance was observed to ampicillin with 88% cumulative resistance. Complete susceptibility was observed to ceftazidime (100%) followed by gentamicin and ciprofloxacin with 92% susceptibility (susceptibility of the isolates to the antibiotics were significantly different at $P < 0.05$). The high resistance pattern by the isolated bacteria against ampicillin in this study supports a study conducted where the antimicrobial susceptibility testing of commensal *Enterobacteriaceae* from free-range chickens using the broth micro-dilution method was done and 89.7% resistance to ampicillin was observed [21]. High percentage resistance may be as a result of the various activities taking place at the various sampling sites (poultry, hostel sewage, cafeteria, cow ranch and abattoir) and the acquisition of respective resistance genes by the different isolated genera.

More importantly, these antibiotics are the easy to buy and abundant pick-over the counter drug in the environment, as a result of over use, most of the organisms in the environment have developed high levels of resistance to the antibiotics.

All the *Enterobacter* isolates were highly sensitive to gentamicin, ciprofloxacin, ofloxacin and nitrofurantoin, this finding is similar to some previous studies in other regions carried out in non-domestic environments [8], [16], [9]. Even though resistance to fluoroquinolones (ciprofloxacin) was not high in this study (8% resistance was observed), over the past decade the emergence of high-level, fluoroquinolone resistance among *E. coli* and other species of *Enterobacteriaceae* has been recorded [23]. Low resistance of all the isolates to gentamicin is supported by previous study where majority of the isolates (over 90%) were sensitive to gentamicin [5]. The most effective antibiotic used in this study was ceftazidime followed by gentamicin, ciprofloxacin, ofloxacin and nitrofurantoin. From this study ten of the isolates were resistant to three or more antibiotics i.e. multiple antibiotic resistance isolates (MAR), four of the isolates were from the cow ranch, three each from the abattoir and the poultry. Presence of isolates with multiple antibiotic resistance at the abattoir supports the result gotten during a study in Nigeria where species of bacteria with potential risk to human health were examined at an abattoir

and it was found that several bacteria such as, *Escherichia coli* O157:H7, *Salmonella* sp. and *Campylobacter* sp. were present in the waste [11]. Several studies in Africa have detected *Salmonella* sp. in wastewater at abattoirs [1], [4], [12]. Pathogens in abattoir waste may originate from the digestive tracts or hides of the animals and most of the pathogens are of enteric sources [10]. Poultry and retail meats are frequently tainted with gastrointestinal flora, which could possibly be foodborne pathogens and high levels of enteric microorganisms are found in the poultry and abattoir environments [15]. Therefore, the spread of foodborne pathogens and resistant bacterial strains from retail meats within the environment is anticipated. This is possible through a pick up process at the slaughter house.

In Nigeria, there is little or no enforcement policy guiding the use of antimicrobials in animals. Consequently, there is a high level of indiscriminate use of antimicrobials in animals [2]. In another study conducted in Maiduguri, located in the arid region of Nigeria, *E. coli* isolates from the tissues of apparently healthy and sick chickens showed resistance to ampicillin (66.7%), chloramphenicol (66.7%), ciprofloxacin (16.7%) and tetracycline (63.3%). These are lower than the rates obtained in a study conducted in Abeokuta, located in the tropical rainforest region of Nigeria [19]. According to [22] & [25] prolonged exposure to therapeutic doses of antimicrobial agents (in plants, animals and humans) is the primary cause of antimicrobial resistance and the use of antibiotics in food animals could enhance the development of antibiotic resistance and its transfer to human pathogens [6]. It is also documented that the use of antimicrobials in agriculture can potentially pilot to extensive diffusion of antimicrobial resistant bacteria [13]. Consumers should therefore avoid the consumption of raw meats and cross-contamination of foods during food handling and preparation. All these will influence the overall prevalence of antimicrobial resistant bacteria within an ecosystem. It is therefore important to educate the general public on effective procedures to sanitize kitchen surfaces, utensils, and hands, especially after handling raw meat, a premise that has also been supported by [14] & [20].

5. Conclusion

The presence of antibiotic resistance genes in isolates from different sources in Iwo, Nigeria, might affect the control of antibiotic-resistant bacteria and make it a little difficult. Thus multiple antibiotic resistances in coliform bacteria in the community continues to be a major health concern.

Indiscriminate use of drugs, improper personal hygiene, lack of knowledge on the usage and consequences of antibiotics are but a few causes of multi antibiotic resistance leading to increase mortality rate in the community and country as a whole. Proper awareness on the use of antibiotics, adequate personal hygiene and essential food processing practices will help reduce the menace and effects of multiple-antibiotic resistance.

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