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### Antibiotic resistance pattern of bacteria spp isolated from hospital waste water in Ede South Western, Nigeria

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#### ABSTRACT

*This study was carried out to investigate the presence of antibiotic resistance bacteria in hospital waste water collected from selected private hospitals in Ede, Southwestern, Nigeria. The bacteria isolates were identified to be Enterobacter aerogenes, Pseudomonas putida, Pseudomonas fluorescens, Klebsiella edwardsii, Proteus mirabilis, Pseudomonas aeruginosa, Shigella spp and Flavobacterium meningosepticum. Antibiotic susceptibility of the bacteria isolates was assayed according to the Kirby – Bauer disc diffusion method. The result of this study indicated that strains of bacteria isolated in this study have become resistant to all the tested antibiotics, and this showed that they have become multi-resistant to these therapeutic agents. This is obvious from the results of percentage of the isolates that are resistant to all the tested antibiotics, from the results it was observed that Enterobacter aerogenes, Pseudomonas putida, Pseudomonas fluorescens, Klebsiella edwardsii, Proteus mirabilis, Pseudomonas aeruginosa, Shigella spp and Flavobacterium meningosepticum were all 100% resistant to Septrin (30µg), Chloramphenicol (30µ), Amoxicillin (30µg) and Streptomycin (30µg) while they were also 90% resistant to Pefloxacin (10µg), Tarivid (30µg), 80% resistant to Ciprofloxacin (10µg) 70% resistant to Gentamycin (10µg). This result showed that these organisms have been well exposed to the tested antimicrobials and they have developed mechanisms to avoid them. This study showed that antibiotic resistant bacteria are also present in Hospitals where people go to for the treatment of infections.*

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## INTRODUCTION

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century (Alanis, 2005). The greatest threat to the use of antibiotics is the emergence and spread of resistance in pathogenic bacteria that consequently cannot be treated by previously successful regimens (WHO, 1997).

There is no doubt that the use of antibiotics provides selective pressure that result in antibiotic resistant bacteria and resistance genes. While some resistant bacteria are found naturally in the environment, pathogens and non pathogens are released into the environment in several ways, contributing to a web of resistance that includes humans, animals, and the environment, essentially the biosphere. Throughout the world the incidences of antimicrobial resistance have been increasing and in such aspect cross-resistance and multi-resistance patterns have been observed (WHO, 2002). The emergence and the development of antimicrobial resistance in micro-organism with its scattering nature therefore turned into a global public health concern.

Many laboratory researches have shown that hospitals and/or health centers that are supposed to be a place where people can go to for cure of infectious diseases have become a breeding place for antibiotic resistant bacteria in Nigeria and other developing countries and this is absolutely worrisome because the effectiveness of antibiotics for medical applications declines, infections once easily curable are now regarded as a growing threat from the drug-resistant microbial agents of these diseases.

Indiscriminate use of antibiotics for medical purposes has taken the brunt of the blame, namely, use by those physicians who prescribe antibiotics for viral infections to make their patients feel comfortable when antibiotics are known to be useless against viruses. In fact, all antibiotic use, whether medical, agricultural, and necessary or not, leads to increased resistance. Studies on antibiotic residues in hospital effluent and in other environmental niches have been conducted mostly in high-income countries, while studies in low- and middle-income settings are few and sparsely distributed and only few study has estimated antibiotic residues in hospital effluents.

It is extremely crystal clear that, concurrent studies on antibiotic prescription quantity in a hospital, antibiotic residue levels in it wastewater and resistant bacteria in the effluent of the same hospital are few. This study was primarily carried out to investigate the presence of antibiotic resistant bacteria in hospital waste water collected from selected private hospitals in Ede, Southwestern, Nigeria.

## MATERIALS AND METHODS

### **Isolation and identification of Bacteria isolates**

The isolation of bacteria was completed within 24 hours of samples collection. This was carried out by mixing 1mL of the waste water sample with 9mL of sterile distilled water and diluted serially up to  $10^{-10}$ . This was repeated for all the water samples. 0.2mL (aliquot) of the suspension was plated out of Mueller – Hinton agar that has been supplemented with 50 $\mu$ g/mL of Septrin respectively. The plates were incubated 35<sup>0</sup>C for 24 hours. Distinct colonies growing

on each plate were counted selected, subcultured and stored on slants. Pure cultures of all the isolates were subjected to biochemical tests.

### Antibiotic Sensitivity Test

Antibiotic susceptibility of the bacteria isolates was assayed according to the Kirby – Bauer disc diffusion method (Bauer *et al.*, 1996). All the plates were incubated for 20 minutes before inoculation and placement of antibiotic disc to allow excess moisture to dry. After the drying, a single loop of each isolate was inoculated into sterile normal saline and compare with 0.5 McFarland standard, the suspension was aseptically swabbed on the surface of Mueller – Hinton plates and antibiotic sensitivity disc that contains Septrin (30µg), Chloramphenicol (30µ), Sparfloxacin (10µg), Ciprofloxacin (10µg), Amoxicillin (30µg), Augmentin (25µg), Gentamycin (10µg), Pefloxacin (10µg), Tarivid (30µg) and Streptomycin (30µg) was aseptically laid on the surface of plates. The plates were incubated at 35°C for 24 hours. After the incubation, zone of growth of inhibition around each disc was measured and used to classify the organisms as sensitive or resistant to an antibiotic according to the interpretive standard of the clinical and Laboratory standards institute (CLSI).

## RESULTS AND DISCUSSION

The bacteria isolates were identified to be *Enterobacter aerogenes*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Klebsiella edwardsii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Shigella spp* and *Flavobacterium meningosepticum*(Table 1).

**Table 1: Results of Biochemical Test**

| ISOLATE LAB. CODE | PROBABLE IDENTITY                     |
|-------------------|---------------------------------------|
| SS                | <i>Enterobacter aerogenes</i>         |
| ST                | <i>Pseudomonas fluorescens</i>        |
| VV                | <i>Pseudomonas putida</i>             |
| UU                | <i>Proteus mirabilis</i>              |
| CC                | <i>Klebsiella edwardsii</i>           |
| HH                | <i>Flavobacterium meningosepticum</i> |
| HI                | <i>Klebsiella edwardsii</i>           |
| AA                | <i>Pseudomonas aeruginosa</i>         |
| ZZ                | <i>Klebsiella edwardsii</i>           |
| YY                | <i>Enterobacter aerogenes</i>         |
| LL                | <i>Enterobacter aerogenes</i>         |
| AC                | <i>Pseudomonas aeruginosa</i>         |
| AZ                | <i>Pseudomonas aeruginosa</i>         |
| GG                | <i>Shigella spp</i>                   |

The result of this study indicated that strains of bacteria isolated in this study have become resistant to all the tested antibiotics, and this showed that they have become multi-resistant to these therapeutic agents, thus rendering these drugs ineffective as treatments of choice for infections caused by these pathogens . This is obvious from the results of percentage of the isolates that are resistant to all the tested antibiotics.

From the results it was observed that *Enterobacter aerogenes*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Klebsiella edwardsii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Shigella spp* and *Flavobacterium meningosepticum* were all 100% resistant to Septrin (30µg), Chloramphenicol (30µ), Amoxicillin (30µg)and Streptomycin (30µg) while they were also 90% Pefloxacin (10µg), Tarivid (30µg) ,80% resistant to Ciprofloxacin (10µg) 70% resistant to Gentamycin (10µg). This result showed that these organisms have been well exposed to the tested antimicrobials and they have developed mechanisms to evade or avoid these antibiotics (Fig.1) .

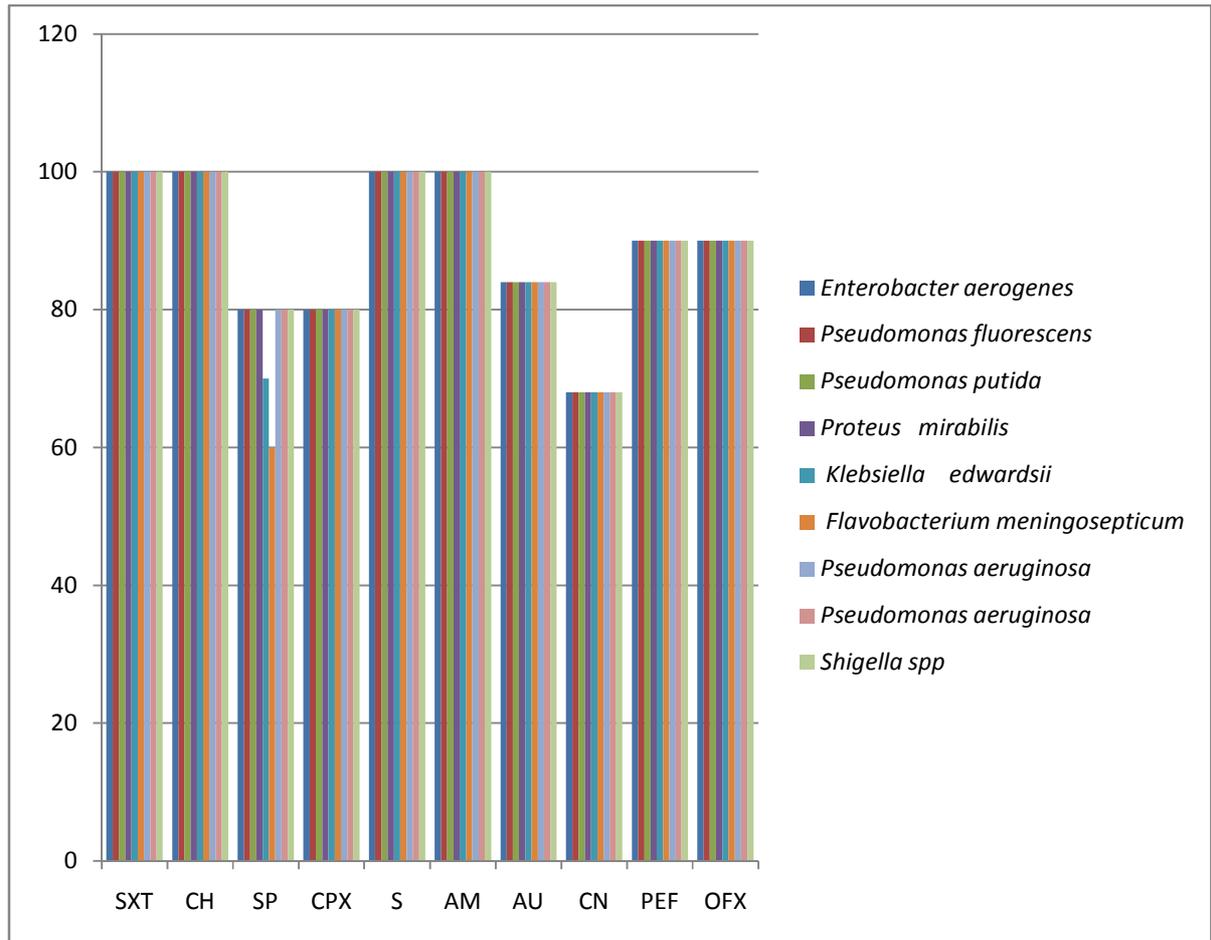


Fig.1: Antibiotic resistance pattern and percentage resistivity.

The presence of antibiotic resistant bacteria in water sources throughout the world has been documented (Kelch and Lee, 1978; Ogan and Nwiika, 1993; Young, 1993). The use of antibiotics in medicine and veterinary practice has aroused some concern about the incidence and spread of antibiotic resistance among bacteria populations. As a result of the usage of antibiotics in medical or veterinary practice, selected for resistant bacteria, these bacteria have inevitably entered the naturally environment. This is particularly true when transfer occurs in environments such as hospitals where the human population is at risk (Jones *et al.*, 1986).The use, misuse and

under-use of antibiotics are responsible for resistance development to bacterial antimicrobials worldwide.

Lateef (2004) reported that in developing countries, drugs are available to the public and thus people may practice self – administration of antibiotics and further increase the prevalence of drug resistant strains. There have been many surveys of the occurrence of antibiotic resistant *E. coli* in animals (Matyar *et al.*, 2004). Chong *et al.*, (1990) found that 204 of 400 faecal samples from human sources containing *E. coli* were resistant to one or more antibiotics at a rate of 83%.

The relatively high level of resistance to antimicrobial agents recorded in this study is a reflection of misuse or abuse of these agents in the environment. Multiple drug resistance is an extremely serious public health problem and it has been found associated with the outbreak of major epidemic throughout the world. Thus, the multiple – drug resistance shown by these pathogens are worrisome and of public health concern (Lateef, 2004).

Encountering multiple antibiotic resistant bacteria in this study is therefore not a surprise but worrisome. Therefore, the occurrence of multiple antibiotic resistant pathogenic bacteria encountered in this study represents a well-known phenomenon that carries a negative impact for public health, an observation that it is in consonance with the reports of Torogolu *et al.*, (2005).

In this study the percentage of antibiotic resistant bacteria recorded for all the tested antibiotic is dreadful because all the isolates were resistant to almost all the antibiotics that are commonly use in the medicine and agriculture for prevention and treatment of infections. These bacteria, like *Pseudomonas aeruginosa*, are common environmental organisms, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised (Lyczak *et al.* 2000).

Because of the prevalent of multiple antibiotic resistant bacteria search for new antibiotics effective against multi-drug resistant pathogenic bacteria is presently an important area of antibiotic research.

## CONCLUSION

This study showed that antibiotic resistant bacteria are also present in Hospitals where people go to for the treatment of infections. This showed that antibiotic resistant bacteria are now everywhere. The pattern of resistant shown by these isolates is in line with the type of antibiotics commonly used in these hospitals. Therefore, government at all tiers should endeavour to sponsor researches on development of new antibiotics that could be relevant in the treatment of severe infections caused by antibiotic resistant bacteria.

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