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Full Length Research Paper

Multiple Antibiotic Resistance among Bacteria Isolated from Hospital Environment

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ABSTRACT

The present study aimed at studying the antibiotics profiles and drug resistance pattern of clinical isolates before and after plasmid curing to find out whether the resistance to multiple antimicrobials was plasmid borne. *Staphylococcus aureus* and *Escherichia coli* had 26.1% and 12.6% incidence of occurrence respectively while lowest incidence of occurrence was observed with *Streptococcus* sp (4.5%). *Staphylococcus aureus* was resistant to streptomycin (44.8%), erythromycin (5.2%), tetracycline (17.2%), gentamycin (15.5%) and cloxacillin (10.3%) while *Klebsiella aerogenes* were resistant to amoxicillin (86.9%), amoxicillin-clavulanic acid (73.9%), and gentamicin (39.1%). All isolated *E. coli* and *Proteus mirabilis* were resistant to amoxicillin-clavulanic acid while resistance to amoxicillin was 71.4% and 2.2% respectively. *Klebsiella aerogenes* was cured of its resistance to nalidixic acid, gentamycin and tetracycline but still retained it resistance to augumetin (17.7%) and amoxicillin (35.0%). All resistant isolated tested against nalidixic acid and gentamycin was observed to be sensitive after plasmid curing while *Pseudomonas aeruginosa* and *E. coli* was observed to be cured of of tetrecycline resistance (66.7%).
Keywords: Plasmid curing, Resistance, Antibiotics, gentamycin, amoxicillin-clavulanic acid

INTRODUCTION

Drug resistance is an alarming problem worldwide and it is spreading rapidly due to overuse, self medication, and non-therapeutic use of antimicrobials (Slama *et al*., 2005). Antimicrobials themselves act as a selective pressure which allows the growth of resistant bacteria within a population and inhibits susceptible bacteria (Levy, 1994). Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). However, the rate of development of resistance appears to have accelerated in the past decade and today multiple resistant bacteria constitute a global problem (Obrien, 1997; Shanahan *et al*., 1994). Hospitalized patients are at higher risk of infections as the hospital environment favors the acquisition of resistance to antimicrobial agents thereby complicating the treatment of nosocomial infections due to antibiotic-resistant pathogens.

Resistance to antibiotics is due to resitance genes which are often located on extrachromosomal genetic elements or in segments inserted within the chromosome that originates from other genomes (Rooklidge, 2004). The acquisition of a new gene may occur by genetic transformation, but when resistance genes are located on plasmids, they can be mobilised by conjugative transfer. The latter may occur at high frequency and efficiency, and several resistance genes can be acquired simultaneously (Rooklidge, 2004). The presence of antibiotic resistance genes on bacterial plasmids has further helped in the transmission and spread of drug resistance among pathogenic bacteria. These plasmids may confer resistance to major classes of antimicrobials, including β-lactams, aminoglycosides, tetracyclines, chloramphenicol, sulfonamides, trimethoprim and quinolones.

Antibiotics resistance costs money and threatens the effectiveness of health delivery programmes (Kim 2000, Jalalpoor *et al.*, 2009). In this study, the antibiotics resistant clinical isolate from two medical facilities were studied before and after plasmid curing to ascertain if the resistance to antibiotics was plasmid borne.

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MATERIALS AND METHODS

Sample Collection

Nutrient agar (BDH, India) and MacConkey agar plates (Oxoid, UK) plates were exposed in six (6) major wards which includes the female medical ward, male medical ward, female surgical ward, male surgical ward, maternity ward and pediatric ward of Faith Mediplex hospital and Central hospital, Benin City, Edo State after which the plates were then taken to the laboratory and incubated at 37^{o}C for 24 hours. All isolates obtained were identified by cultural, morphological and biochemical tests.

Antibiotic sensitivity assay of bacterial isolates

Bacteria isolates were screened for resistance to antibiotics (Oxoid, UK) using the Kirby Bauer standardized agar disc-diffusion method (NCCLS, 2002). Antibiotic agents tested were; gentamycin (10µg), augumentin (30µg), streptomycin (10µg), nalidixic Acid (30µg), erythromycin (5µg), cloxacillin (5µg), amoxicillin (10g), and tetracycline (10µg). The plates were incubated at 37°C over night. The zones of inhibition were recorded in millimeter (mm) and the isolated classified as resistant or sensitive based on the interpretative chart updated according to the current NCCLS Standards.

Plasmid curing

Plasmid curing was carried out on organisms that were resistant to minimum of 3 antibiotics using sub-inhibitory concentration of 10% of sodium dodecyl sulphate (SDS) as described by Sheikh *et al*. (2003) and Yah *et al.* (2008) with slight modification. Antibiotic resistant isolates were grown on nutrient broth containing 10% SDS at 37^{o}C for 48hrs. After 48hrs, the broth was agitated to homogenize the content and a loopful subcultured onto Mueller Hinton agar (MHA) plates. The plates were incubated at 37ºC for 24 hours after which colonies were screened for antibiotic resistance by the disk diffusion method. Cured markers were determined by comparison between the pre- and post- curing antibiograms of isolates. Loss of resistance after the plasmid curing was indicative of plasmid mediated resistance.

RESULT AND DISCUSSION

The bacteria isolated from all the hospital wards were identified as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella aerogenes, and Streptococcus* sp (Table 1). *Staphylococcus aureus* and *Escherichia coli* had 26.1% and 12.6% incidence of occurrence respectively while lowest incidence of occurrence was observed with *Streptococcus* sp (4.5%).

The presence of these genera of bacteria in indoor air of hospitals is known to cause nosocomial infections. burge and hoyer, (1990) reported these genera of bacteria to be the most common bacterial often isolated from the air. Also, Ekhaise *et al*., (2008*)* isolated similar genera of bacteria from Faith mediplex and Central hospital in Benin city. It can be seen that *Staphylococcus aureus* had the highest incidence of occurrence. Staphylococcal count constituting 50-71% of the total bacterial genera was reported by Pastuszka *et al*., (2005) and Nevalainen (1989). The microbial flora of the indoor air depends on several factors including the number and hygienic standard of people present, the quality of the hospital system and human activities such as talking, walking, laughing, sweeping and coughing. Similar results were reported by Okhuoya and Okaraedje (1992).

Table 1: Incidence of bacterial isolates from hospital (%)

Bacterial isolates	No. of isolates (%)
Pseudomonas aeruginosa	35 (15.8)
E. coli	28 (12.6)
Staphylococcus aureus	58 (26.1)
Bacillus subtilis	50 (22.5)
Klebsiella aerogenes	23 (10.4)
Proteus mirabilis	18 (8.1)
Streptococcus sp	10 (4.5)
	222 (100.0)

Majority of the organisms isolated showed multiple drug resistance to the drugs tested. From the resistance pattern it was found that all *Pseudomonas aeruginosa* isolated was resistant to augumentin while 71.4% was resistant to amoxicillin and 40% was resistant to nalidixic acid (Table 2). *Klebsiella aerogenes* were resistant to amoxicillin (86.9%), Augumentin (73.9%), and gentamicin

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(39.1%). All isolated *E. coli* and *Proteus mirabilis* were resistant to Augumentin while resistance to amoxicillin was 71.4% and 2.2% respectively.

Table 2: Prevalence of antibiotics resistant isolates

	No. of isolates	No. Resistant to Nalidixic acid	No. Resistant to Gentamycin	No. Resistant to Tetracycline	No. Resistant to amoxicillin -clavulanic acid	No. Resistant to Amoxicillin
Pseudomonas aeruginosa	35	14 (40.0)	21 (60.0)	9 (25.7)	35 (100.0)	25 (71.4)
E. coli	28	9 (32.1)	NIL (0.0)	12 (42.9)	28 (100.0)	20 (71.4)
Klebsiella aerogenes	23	8 (34.8)	9 (39.1)	11 (47.8)	17 (73.9)	20 (86.9)
Proteus mirabilis	18	12 (66.7)	6 (33.3)	3 (16.7)	18 (100.0)	13 (72.2)

The resistance pattern of gram positive organisms is shown in Table 3. It was observed that *Bacillus subtilis* was resistant to streptomycin (22%), erythromycin (8%), tetracycline (32%), gentamycin (28%) and cloxacillin (30%) while *Staphylococcus aureus* was resistant to streptomycin (44.8%), erythromycin (5.2%), tetracycline (17.2%), gentamycin (15.5%) and cloxacillin (10.3%). Resistance to antibiotics has been ascribed in most instances to the presence of plasmids (Adeleke and Odelola, 1997; Bhakta *et al*., 2003; Daini *et al*., 2006; Diep *et al*., 2006). These antibiotic resistant properties of a bacterium could be its inherent properties or occurs due to chromosomal mutation(s) or by acquiring extra-chromosomal DNA plasmid (Mandal *et al*., 2004). According Boucher *et al*. (2009), ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) are a group of particularly troublesome bacteria having the ability to resist antibiotics.

Table 3: Prevalence of antibiotics resistant isolates

	No. of isolates	No. Resistant to streptomycin	No. Resistant to Gentamycin	No. Resistant to Cloxacillin	No. Resistant to Erythromycin	No. Resistant to Tetracycline
Staphylococcus aureus	58	26 (44.8)	9 (15.5)	6 (10.3)	3 (5.2)	10 (17.2)
Bacillus subtilis	50	11 (22.0)	14 (28.0)	15(30.0)	4 (8.0)	16 (32.0)
Streptococcus sp	10	4 (40.0)	3 (30.0)	2 (20.0)	1 (0.0)	2 (2.0)

Antibiogram after plasmid curing of resistant isolates is shown in Table 4 & 5. *Klebsiella aerogenes* was cured of its resistance to Nalidixic acid, Gentamycin and Tetracycline but *Klebsiella aerogenes* still retained it resistance to Augumetin (17.7%) and Amoxicillin (35.0%). All resistant isolate tested against nalidixic acid and gentamycin was observed to be sensitive after plasmid curing. *Pseudomonas aeruginosa* and *E. coli* was observed to be cured of 66.7% of Tetracycline resistance after curing. All *Streptococcus* sp were sensitive to streptomycin, gentamycin, erythromycin and tetracycline while 50% of the isolates were still resistant to cloxaccillin after plasmid curing. *Staphylococcus aureus* showed varying degree of sensitivity after plasmid curing. Only 16 (61.5%) were cured of streptomycin resistance, 8 (88.8%) were cured of gentamycin resistance, 1 (11.1%) were cured of cloxacillin resistance, 2 (66.7%) were cured of erythromycin resistance and 8 (80.0%) were cured of tetracycline resistance

Most of the drug-resistant strains carried resistant plasmids from this study. Emergence of multidrug resistance in pathogenic bacteria has created immense clinical problem in the treatment of infectious disease and outbreaks of *S. aureus* resistant to antibiotics have been frequently associated with devastating nosocomial infections (Depardieu *et al*., 2007; Buhlmann *et al*., 2008). Elimination of plasmid DNA mediated antibiotic resistance in pathogenic bacteria is great practical significance both in chemotherapy of bacteria and in microbial genetics. The response of bacterial isolates in different rates to 10% SDS may be related to the permeability through outer membrane and the location of antibiotic resistance genes, while the effectiveness of SDS may be related to plasmid copy number or amount of the enzyme which inactivate antibiotics (Khalid,

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2005). Similar results have been reported by Diep *et al*., (2006) and Han *et al*., (2007) who showed that plasmid determined resistance to amoxycillin, tetracycline, and chloramphenicol. The presence of plasmid mediated resistance for amoxicillin has also been reported by Cattoir *et al.*, (2008). These study demonstrated that the plasmid is one of the important ways to spread resistance but chromosomal mutation by environmental selection might also responsible for resistance.

Table 4: Antibiogram of Plasmid cured resistant isolates

	No. cured of Nalidixic acid Resistance	No. cured of Gentamycin Resistance	No. cured of Tetracycline Resistance	No. cured of Augumentin Resistance	No. cured of Amoxicillin Resistance
Pseudomonas aeruginosa	14 (100.0)	21 (100.0)	6 (66.7)	20 (57.1)	10 (40.0)
E. coli	9 (100.0)	-	8 (66.7)	28 (100.0)	15 (75.0)
Klebsiella aerogenes	8 (100.0)	9 (100.0)	11 (100.0)	14 (82.3)	13 (65.0)
Proteus mirabilis	12 (100.0)	6 (100.0)	3 (100.0)	12 (66.7)	8 (61.5)

Table 5: Antibiogram of Plasmid cured resistant isolates

	No. cured of streptomycin Resistance	No. cured of Gentamycin Resistance	No. cured of Cloxacillin Resistance	No. cured of Erythromycin Resistance	No. cured of Tetracycline Resistance
Staphylococcus aureus	16 (61.5)	8 (88.8)	1 (11.1)	2 (66.7)	8 (80.0)
Bacillus subtilis	8 (72.7)	14 (100.0)	8 (53.3)	3 (75.0)	15 (93.8)
Streptococcus sp	4 (100.0)	3 (100.0)	1(50.0)	1 (100.0)	2 (100.0)

CONCLUSION

It is therefore important to evaluate the quality of air whether indoor or outdoor in hospital environments. The number and type of airborne of airborne microflora in hospital wards can be used to determine of hygiene. There is also need for consistent on-going antimicrobial resistance surveillance for important and commonly isolated clinically significant pathogens to form the basis for developing and implementing measures that can reduce the burden of antimicrobial resistance and prevent a probable impending public health problem.

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