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Full Length Research Paper

Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria

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Because antimicrobial resistance patterns are continually evolving and multi-drug resistant (MDR) organisms undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles will continue to be essential to ensure the provision of safe and effective empiric therapies. This current study reports on the assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. Thirteen antibiotics were bought from different pharmacy shops in Calabar metropolis and their susceptibility profiles were evaluated against some clinical isolates obtained from Microbiology Section of Sufat Medical Laboratories, Ishie, Calabar; Microbiology laboratory Unit of University of Calabar Teaching Hospital, Calabar and Department of Microbiology, University of Calabar, Calabar. These included *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes.* The species level identification was then carried out by standard biochemical test and by comparing their characteristics with those of known taxa. Susceptibility tests were performed by Bauer-Kirby disc diffusion by using Muller Hinton Agar (CM337-Oxoid). The results were expressed as susceptible/resistant according to criteria developed by NCCLS. *S. aureus* was susceptible to 09 (75.0%) and resistant to 3 (25.0%) of 12 antibiotics used. *Str. pyogenes* was susceptible to 8 (66.7%) antibiotics and resistant to 4 (33.3%). Of 11 antibiotics tested against *E. coli* and *P. aeruginosa*, *E. coli* was susceptible to 10 (90.9%) and resistant to 1 (09.1%). *P. aeruginosa* was susceptible to 9 (81.8%) and resistant to 2 (18.2%). Of 13 antibiotics tested against *K. pnenmoniae*, it was susceptible to 10(83.3%) and resistant to 2(16.7%). Resistance to chloramphenicol was common to all isolates except *K. pneumoniae*. Erythromycin- resistance was common to *Str. pyogenes*. Also, rifampicin-resistance was common to *S. aureus*. Resistance to gentamicin and tetracycline was only common to *Str. pyogenes* while penicillin-resistance was common to *S. aureus* only. Though, some multi-drug resistant organisms were reported in this study, some organisms were highly susceptible to most of the test antibiotics. There are several limitations of this work. Nevertheless, the results can serve to direct any national effort aimed toward reducing the antimicrobial resistance problems of local hospitals. The reasons for the differences in antimicrobial drug–resistant patterns might be related to infection control practices or to timing of the introduction of resistant organisms. However, more research is needed to clarify these differences. We believe that our findings represent the endemic multi-drug resistant situation in our hospitals in Nigeria.

Key words: Antibiotics, assessment, clinical isolates, resistance pattern, susceptibility profiles

INTRODUCTION

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antibacterial agents. Antimicrobial resistance is a well-known clinical and public health problem (Oteo et al., 2002; Okonko et al., 2009a). Bacterial antimicrobial drug resistance is a worldwide problem that is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline (Spellberg et al., 2004; Talbot et al., 2006; Okonko et al., 2009a). This is an emerging public health problem, especially in hospitals of the newly industrialized countries of Asia and the Pacific (Hsu et al., 2007; Okonko et al., 2009a).

However, drug resistance has also become a large and growing problem in infections that account for most of Africa's disease burden, including respiratory and diarrheal diseases (Okeke et al., 2007; Okonko et al., 2009b). Much of the current discourse on infectious disease and drug resistance as it affects Sub-Saharan Africa is limited to the pressing problems associated with emerging- and re-emerging resistant organisms. Resistance, however, equally compromises the management of acute respiratory infections, sexually transmitted diseases and diseases spread by the fecal–oral route, such as typhoid fever, cholera, dysentery and other diarrheal diseases (Okeke et al., 2007; Okonko et al., 2009b).

The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections (Nawaz et al., 2009). Resistance based on decreased entry of drugs has been found for penicillins, cephalosporins, aminoglycosides and tetracyclines in the Enterobacteriaceae and *Pseudomonas aeruginosa*. Beta-lactamase resistance has increased significantly being encountered in Neisseria, Haemophilus, Enterobacteriaceae and *Pseudomonas* species (Neu, 1984; Okonko et al., 2009a). Available therapeutic options for antibiotic-resistant organisms are severely limited, as these organisms frequently display a multidrug-resistant (MDR) phenotype (Moland et al., 2006; Lewis et al., 2007; Chikere et al., 2008; Okonko et al., 2009a).

The prevalent organisms that are usually isolated from clinical samples such as urine are *P. aeruginosa* and *Enterobacter* spp. These prevalent microorganisms have been found to be resistant to most chemotherapeutic agents (Okonko et al., 2009a). *Escherichia coli* is one of the main causes of both nosocomial and community-acquired infections in humans (Diekema et al., 1999). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Sahm et al., 2001; Karlowsky et al., 2004; Okonko et al., 2009a). Bacteria of the Klebsiella genus may cause numerous infections in human, which are often treated with beta-lactam antibiotics (Amin et al., 2009).

The fundamental mechanism of *Klebsiella* resistance to penicillin or cephalosporin involves the production of enzymes called extended spectrum - lactamases (ESBLs), because of resistance of many *Klebsiella spp.* strains to -lactamases; alternative antibiotic therapy can make use of aminoglycosides and quinolones (Sekowska et al., 2002; Amin et al., 2009; Okonko et al., 2009a). However, *for Staphylococcus aureus* and *P. aeruginosa*, an increase of resistance has been reported. The underlying mechanisms seem to be unchanged (Okonko et al., 2009a).

Multidrug resistance is frequent, and clinical isolates resistant to virtually all anti-pseudomonal agents are increasingly being reported (Rossolini and Mantengoli, 2005; Okonko et al., 2009a). *P. aeruginosa* is one of the leading causes of nosocomial infections. The ability of *P. aeruginosa* to persist and multiply in moist environments and equipment, such as humidifiers in hospital wards, bathrooms, sinks and kitchens may be of importance in cross-infection. Multi-drug resistant gram-positive bacteria including methicillin-resistant *S. aureus* (MRSA), methicillin- resistant coagulase-negative staphycolocci (MRCNS), penicillin-resistant *S. pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE) have been a serious problem in the medical community (Takahashi et al., 2003; Okonko et al., 2009a). These problems of multi-drug resistance have been the driving force for the development of newer quinolones.

There has been serious concerns regarding increased prevalence of extended spectrum -lactamases (ESBLs) in different parts of the world, although exact prevalence in not known (Amin et al., 2009). ESBLs prevalence rate is 0 to 25% in USA, 0 to 40% in Netherlands, (Stobberingh et al., 1999) 40% in France, (Branger et al., 1998) 4.8% in Korea, (Jarlier et al., 1998) up to 12% in Hong Kong (Tsang et al., 2000) 51% in China (Xiong et al., 2002) 86.6% in India (Jain et al., 2003) and 47.5% in Pakistan (Amin et al., 2009), whereby antibiotic overuse, prescription of drugs with proper sensitivity test and over dosing may have created this problem in developing nations. The *K. pneumoniae* and *E. coli* contribute 10 to 40% of ESBLs (Amin et al., 2009). Multidrug resistance and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to the successful management of disease scourge.

Also, the rising prevalence of drug resistance such as penicillin-resistant pneumococci worldwide mandates selec-tive susceptibility testing and epidemiological investigations during outbreaks (Okonko et al., 2008). However, high ESBLs prevalence not only complicates antibiotic therapy

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but also interfere with empirical therapy resulting in increased morbidity and mortality (Amin et al., 2009). The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment (Okonko et al., 2009a).

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms (WHOCCDSM, 1999; Oteo et al., 2002; Okonko et al., 2009a). The worldwide escalation in both community-and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and new treatment alternatives (Mulvey, 2004; Rhomberg et al., 2006; Chikere et al., 2008; Zhanel et al., 2008; Okonko et al., 2009a).

In 2001, the World Health Organization (WHO) launched the first global strategy to counter this phenomenon (Simonsen et al., 2004), a key component of which is the development of surveillance programs to monitor trends in antimicrobial drug resistance and use (Simonsen et al., 2004; Hsu et al., 2007; Okonko et al., 2009a). Surveillance studies are valuable tool for assessing the changes in pattern of resistance of clinical isolates of antimicrobial agents. Trend towards increased antimicrobial resistance shown by many of the gram negative bacteria is worrying, and has developed as a consequence of widespread and inappropriate use of various agents (Amin et al., 2009). Therefore, this current study reports on the assessment of antibiotics susceptibility profiles of selected clinical isolates from laboratories in Nigeria.

MATERIALS AND METHODS

Bacterial strains

The study includes clinical isolates of *E. coli, Klebsiella pneumoniae*, *P. aeruginosa*, *S. aureus* and *Str. pyogenes* obtained by screening samples of blood (for blood culture), urine, pus, wound, sputum, etc. Clinical isolates were isolated from Microbiology Section of the Sufat Medical Laboratories, Ishie, Calabar, the Microbiology laboratory of the University of Calabar Teaching Hospital, Calabar and the Department of Microbiology, University of Calabar, (UCTH), Calabar respectively. University of Calabar Teaching Hospital provides a tertiary level patient care and equipped with capacity of 100s of beds with medical and surgical specialties. It serves as referral hospital in Calabar and provides health care facilities to people of different areas.

Initially strains were identified based on the morphological behavior of the isolates on various differential media. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 mm at 15 lb pressure. The species level identification was then carried out by standard biochemical test (Bergey's Manual of Determinative Bacteriology ninth edition) and by comparing their characteristics with those of known taxa, as described by Jolt et al. (1994), Cheesbrough (2006) and Oyeleke and Manga (2008).

Antibiotic susceptibility testing

Susceptibility tests were performed by Bauer-Kirby (Bauer et al., 1966) disc diffusion by using Muller Hinton Agar (CM337-Oxoid). The results were expressed as susceptible/resistant according to criteria developed by National Committee for Clinical Laboratory Standards (NCCLS, 2002) and Manual of Antimicrobial Susceptibility Testing guidelines (Coyle, 2005; Cheesbrough, 2006; Okonko et al., 2009a, b). The standard commercial disks with their concentrations were as follows: Amoxicillin (25 mg), Ampicillin (25 mcg), Ampiclox (10 mcg), Chloramphenicol (25 mcg), Ciprofloxacin (10 mcg); Erythromycin (10 mcg), Fulcin (10 mcg), Gentamicin (10 mcg), Penicillin G (10 mcg), Rifampicin (10 mcg), Septrin (25 mcg), Tetracycline (25 mcg), Vancomycin (10 mcg).

Trypticase soy broth (BBL™ Trypticase™ Soy Broth, BIOTECH) was prepared. Five discrete colonies of the different identified isolates were inoculated into 5 ml of the broths and incubated at 35°C for 4 – 6 h. The inoculum for primary sensitivity testing was prepared from a broth that has been incubated for 4 – 6 h. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standard. Each of the isolates was uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method using sterile cotton wool. A sterile cotton wool was allowed to soak in the broth culture, squeezed by the side of the bottle before streaking over the sensitivity plates. The appropriate antibiotic multi -discs (either Gram positive or negative) were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37°C for 24 h. The standard positive commercial disks included gram positive, gram negative and broad spectrum disks while the negative control disks were impregnated with sterile distilled water. For *Str. pyogenes,* Mueller-Hinton agar was used. After incubation, the zones of clearance of organisms around the disks were also measured and recorded (Baker et al., 2001; NCCLS, 2002; Coyle, 2005; Cheesbrough, 2006; Okonko et al., 2009a, b). Multidrug resistance was defined as resistance to 3 of the antimicrobial agents tested (Oteo et al., 2005).

RESULTS

The isolates used were confirmed to be *E. coli, K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *Str. pyogenes*. The antibiotics susceptibility profiles of these test bacteria are presented in Tables 1 to 2. The antibiotics susceptibility profiles of these organism also differ among the test antibiotics used.

Table 1 shows the antibiotics susceptibility profiles of gram positive bacteria. From the results, all gram positive bacteria were susceptible to amoxicillin, ampicillin, ampiclox, ciprofloxacin, septrin and vancomycin with 100% inhibitory activity and resistant to chloramphenicol with 100% resistivity (Table 1). Among the gram positive isolates, *S. aureus* was susceptible to 09 (75.0%) and resistant to 3 (25.0%) of 12 antibiotics used. *Str. pyogenes* was susceptible to 8 (66.7%) antibiotics and resistant to 4 (33.3%). Both showed some degree of resistance to some of the test antibiotics. *S. aureus* was resistant to chloramphenicol, penicillin and rifampicin. *Str. pyogenes* was resistant to chloramphenicol, erythromycin, gentamicin and tetracycline as shown in Table 1.

Table 2 shows the antibiotics susceptibility profiles of gram negative bacteria. From Table 2, all gram negative

Table 1. Antibiotics susceptibility profiles of gram positive isolates.

S/n	Antibiotics (mcg)	*S. aureus*	*Str. pyogenes*
1	Amoxicillin (25)	S	S
2	Ampicillin (10)	S	S
3	Ampiclox (10)	S	S
4	Chloramphenicol (25)	R	R
5	Ciprofolxacin (10)	S	S
6	Erythromycin (10)	S	R
7	Gentamicin (10)	S	R
8	Penicillin G(10U)	R	S
9	Rifampicin (10)	R	S
10	Septrin (25)	S	S
11	Tetracycline (25)	S	R
12	Vancomycin (10)	S	S
	No. Sensitive (%)	09 (75.0)	08 (66.7)
	No. Resistant (%)	03 (25.0)	04 (33.3)

Key: S = Susceptible, R = Resistant.

Table 2. Antibiotics susceptibility profiles of gram negative isolates.

S/n	Antibiotics (mcg)	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
1	Amoxicillin (25)	S	S	S
2	Ampicillin (10)	S	S	S
3	Ampiclox (10)	S	S	S
4	Chloramphenicol (25)	S	S	R
5	Ciprofolxacin (10)	S	S	S
6	Erythromycin (10)	NA	S	S
7	Gentamicin (10)	S	S	S
8	Penicillin G (10U)	S	S	S
9	Rifampicin (10)	S	S	NA
10	Septrin (25)	S	S	S
11	Tetracycline (25)	S	R	S
12	Vancomycin (10)	R	R	R
	No. Sensitive (%)	10 (90.9)	10 (83.3)	09 (81.8)
	No. Resistant (%)	01 (09.1)	02 (16.7)	02 (18.2)

Key: S = Susceptible, R = Resistant, NA = Not applicable.

bacteria were susceptible to amoxicillin, ampicillin, ampiclox, ciprofloxacin, gentamicin, penicillin and septrin with 100% inhibitory activity and resistant to vancomycin (100%). Of 11 antibiotics tested against *E. coli* and *P. aeruginosa* , *E. coli* was susceptible to 10 (90.9%) and resistant to 1 (09.1%). *P. aeruginosa* was susceptible to 9 (81.8%) and resistant to 2 (18.2%). Of 13 antibiotics tested against *K. pnenmoniae*, it was susceptible to 10 (83.3%) and resistant to 2 (16.7%). Resistance to chloramphenicol was common to all isolates except *K. pneumonia* (Table 2).

E. coli was resistant to vancomycin. *K. pneumoniae* was resistant to tetracycline and vancomycin. *P. aeruginosa* was resistant to chloramphenicol and vancomycin as shown in Table 2.

DISCUSSION

This susceptibility assessment measures the ability of the different antibiotics to inhibit bacterial growth. In assessing the antibiotics susceptibility profiles of some selected clinical isolates from different laboratories in Nigeria; *S. aureus* was susceptible to 09 (75.0%) and resistant to 3 (25.0%) of 12 antibiotics used. *Str. pyogenes* was susceptible to 8 (66.7%) antibiotics and resistant to 4 (33.3%). *E. coli* was susceptible to 10 (90.9%) and resistant to 1 (09.1%). *P. aeruginosa* was susceptible to

9 (81.8%) and resistant to 2 (18.2%). *K. pnenmoniae* was susceptible to 10 (83.3%) and resistant to 2 (16.7%). From the study, the degree of susceptibilities shown by these antibiotics against the test organisms indicates their potencies (Pelczar and Reid, 1998; Cheesbrough, 2006). The susceptibility of a chemotherapeutic agent is usually expressed on the basis of the lowest concen-tration of MIC or higher zones of inhibition (Nnela and Cox, 1998).

Antibiotics susceptibility tests revealed that all the bacterial pathogens were susceptible to amoxicillin, ampicillin, ampiclox, ciprofloxacin and septrin (cotrimoxazole) in equal magnitude (100%). However, the pathogens tested in this study showed varying degrees of resistance (1 to 4 of the antibiotics). All tested bacteria were resistant to vancomycin, except for *S. aureus* and *Str. pyogenes* which were susceptible to vancomycin. Resistance to chloramphenicol was common to all isolates except *K. pneumoniae*. Erythromycin-resistance was common to *Str. pyogenes*. Also, rifampicin-resistance was common to *S. aureus* and resistance to gentamicin and tetracycline was only common to *Str. pyogenes* while penicillin-resistance was common to *S. aureus* only.

The findings of this present study is a deviation from what was recently reported by Okonko et al. (2009a), who reported 100% resistance to ampicillin by all isolates and 100% resistance to septrin (cotrimoxazole) by gram negative isolates and 50% by gram positive isolates. Okonko et al. (2009a) reported all gram positive isolates to be 100% resistant to gentamicin. Okonko et al. (2009a) also reported 100% resistant to chloramphenicol and tetracyline by all isolates and that all isolates were more resistant to cotrimoxazole and gentamycin in a recent study. Alos et al. (1993) and Oteo et al. (2002) reported 27% and 30% resistance to septrin (cotrimoxazole) respectively in a similar study. Aiyegoro et al. (2007) reported that 66.7% of the pathogens were resistance to septrin (cotrimoxazole) while Christiaen et al. (1998) reported a resistance of 17% to septrin (cotrimoxazole) in their study.

The 100% susceptibilities to gentamicin by gram negative bacteria in this study is comparable to what was reported by Chikere et al. (2008) who reported the similar sensitivities of gram negative isolates to gentamicin. Chikere et al. (2008) also reported susceptibility of gram positive isolates to gentamicin to be 93.3%; this is a deviation to our present finding. Susceptibility of gram positive bacteria to gentamicin in this study was 50%. This is also higher than what was reported in a similar study by Onifade et al. (2005), Aiyegoro et al. (2007) and Okonko et al. (2009a). Susceptibility to ciprofloxacin reported in this study was 100%. This is similar to that reported by Reish et al. (1993) and Okonko et al. (2009a) and deviate from the 63.6% reported by Chikere et al. (2008) in a similar study. Aiyegoro et al. (2007) reported 77.8% sensitivity to Ciprofloxacin. Susceptibility to septrin (cotrimoxazole) by all isolates in this study was quite high (100%) compared to those commonly reported. Okonko et al. (2009a) reported 100% resistance in a similar study.

Our present study highlights the most alarming situation of highly diverse antibiotics resistance rates against most antibiotics ranging from 9.1 to 33.0%. No doubt this represents very critical situation as compared to investigations from other regions of world reporting resistance towards first, second and third generation antibiotics (Reish et al., 1993; Gupta et al., 1999; Olowu and Oyetunji, 2003; Fagade et al., 2005; Onifade et al., 2005; Aiyegoro et al., 2007; Hsu et al., 2007; Chikere et al., 2008; Amin et al., 2009; Okonko et al., 2009a, b). This may be due production of ESBLs which cause the hydrolysis of -lactam ring resulting in inactivation of penicillin antibiotics (Yano et al., 2001) and over use of antibiotics in developing nations resulting in high levels of resistance (Amin et al., 2009).

In our present study, *S. aureus* showed marked susceptibility to 9 (75.0%) and resistant to 3 (25.0%) antibiotics (chloramphenicol, penicillin and rifampicin). Marked resistance to ampiclox by *S. aureus* was reported in a study Okonko et al. (2009a) and Hsu et al. (2007) also reported that Methicillin-resistant *S. aureus* (MRSA) strains showed correspondingly high resistance levels to ciprofloxacin (93.9%). These also deviate from the finding of our present study as no *S. aureus* was reported to be resistant to ampiclox and ciprofloxacin. Penicillin and chloramphenicol resistant *S. aureus* was also reported in this study.

This is comparable to what has been previously reported. *S. aureus* has been reported to exhibit resistance to beta-lactam antibiotics of which benzyl penicillin is one. Outbreaks of *S. aureus* resistant to beta-lactam antibiotics have been frequently associated with devastating nosocomial infections (Depardieu et al., 2007; Buhlmann et al., 2008; Chikere et al., 2008). Also, *Str. pyrogenes* showed varying degrees of resistance to 4 (33.3%) of the antibiotics used in this study. Similar degree of resistance was also reported by Chikere et al. (2008) and Okonko et al. (2009a).

In this study, *K. pneumoniae* showed a high level of susceptibility to most of the antibiotics [10 (83.3%)] used and resistant to tetracycline and vancomycin. This is a deviation to what was previously reported (Reish et al., 1993; Aiyegoro et al., 2007; Okonko et al., 2009a). Reish et al. (1993) and Aiyegoro et al. (2007) reported resistance of 66.7% against amoxicillin and cotrimoxazole and 55.6% to tetracycline by *Klebsiella* spp. In an outbreak of multi-resistance *Klebsiella* in a neonatal intensive care unit in a hospital in Israel, *Klebsiella* isolates were reported to be resistant to chloramphenicol and gentamycin (Reish et al., 1993), this also deviate from our present finding.

A high level of resistance to most of the antibiotics by *Klebsiella* spp. was reported by Okonko et al. (2009a) this is contrary to our present finding. *K. pneumoniae* iso-

lates resistance to ciprofloxacin and amoxicillin/clavulanic acid was reported to 55% and 12.5% in a study by Amin et al. (2009). There are reports covering high levels of resistance of *K. pneumoniae* towards these antibiotics in many countries (Subha and Ananthan, 2002). This is also contrary to our present study. Moreover limited use of these antibiotics is one of low levels of resistance towards *K. pneumoniae* (Amin et al., 2009).

E. coli was susceptible to 10 (90.9%) of the test antibiotics and resistant to 1 (09.1%) antibiotics (erythromycin and vancomycin) . This is in contrast with what was reported by Hsu et al. (2007), who reported that *K. pneumoniae* and *E. coli* to be resistant to ciprofloxacin in their study on antimicrobial Drug Resistance in Singapore Hospitals. Susceptibility to tetracycline by *E. coli* reported in this study agrees favorably with Gupta et al. (1999) and Aiyegoro et al. (2007) who reported sensitivity of *E. coli* to tetracycline. Also, susceptibility to septrin (cotrimoxazole) by *E. coli* reported in this study is in agreement with results obtained elsewhere and in contrast to 100% resistance reported by Okonko et al. (2009a).

P. aeruginosa showed resistant to 2 (18.2%) antibiotics *in vitro* (chloramphenicol and vancomycin). This is also comparable to the findings of Okonko et al. (2009a) who reported multi-drug resistant (MDR) to 5 antibiotics (ampicillin, chloramphenicol, cotrimoxazole, nitrofuratoin and tetracycline) by *P. aeruginosa* and deviate slightly in that only resistant to chloramphenicol and no resistance to ampicillin, cotrimoxazole, and tetracycline was observed in our study. Multi-resistance *P. aeruginosa* was also isolated by Olowu and Oyetunji (2003) in their study of nosocomial urinary tract infection and Fagade et al. (2005) in their study on clinical samples. Aiyegoro et al. (2007) also isolated multi-resistance *P. aeruginosa* in their study to determine the incidence of urinary tract infection in children and adolescents in Ile- Ife.

The pattern of multi-drug resistance of 1 - 4 antibiotics was frequent (9.1 - 33.3%) . This is comparable to the previous findings (Okonko et al., 2009a). Multi-drug resistance in the United States among 38,835 urinary tract infection isolates was 7.1% in 2000 (Sahm et al., 2001). Such multi-drug resistance has important implications for the empiric therapy of infections caused by *Klebsiella* spp., *P. aeruginosa*, *E. coli, S. aureus* and *Str. pyrogenes* and for the possible co-selection of antimicrobial resistance mediated by multi- drug resistance plasmids (Oteo et al., 2002; Sherley et al., 2004). It is also well documented that gram negative bacilli habour series of antibiotic resistant genes which can be transferred to other bacteria horizontally (Piddock, 2006; Depardieu et al., 2007; Leavitt et al., 2007; Lockhart et al., 2007).

Bacterial resistance to beta- lactam antibiotics is primarily due to the production of betalactam ring of the antibiotics rendering them inactive (Akpan, 1992). Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates (Chikere et al., 2008; Prescott et al., 2008) . In developing countries like Nigeria, self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable to treat themselves.

The community acquired resistant strains on admission exchange genetic information with nosocomial isolates resulting in the emergence of 'super bugs' that could cause difficult- to-treat infections (Mulvey et al., 2004; Chikere et al., 2008).The worldwide escalation in both community- and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control and new treatment alternatives (Mulvey et al., 2004; Rhomberg et al., 2006; Zhanel et al., 2008; Chikere et al., 2008). The use of molecular biology techniques would also enhance the molecular identification of resistance genes (Singh et al., 2006; Turnidge and Paterson, 2007; Chikere et al., 2008).

The study reveals the prevalence of multi-drug-resistant *E. coli, K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *Str. pyrogenes* in the environment; hence caution must be exercised whenever antibiotics therapy is to be administered. This study shows a high level of multi-drug resistance to chloramphenicol, erythromycin, gentamicin, penicillin, rifampicin, tetracycline and vancomycin in equal magnitude *in vitro* and as such, these antimicro-bials may not be suitable for treating case of nosocomial or community acquired infection in this locality (Okonko et al., 2009a, b). Indeed, the problem of antibiotic resistance is global. An organism's expression of a novel gene coding for drug resistance in remote communities has implications for the developed world. Once a resistant organism is introduced into a population, it is rapidly disseminated (Okonko et al., 2009a).

Though, some multi- drug resistant patterns were reported among bacteria tested in this study, most of the test bacteria highly susceptible. Resistance due to over use and adulteration of the antibiotics has also been reported (Pelczar and Reid, 1998; Cheesbrough, 2006). And the implication these resistance, is that many bacterial and parasitic diseases that could, until recently, be treated with inexpensive antimicrobial agents, has recently been made more expensive and less successful by the emergence and spread of resistant organisms (Okeke et al., 2007; Okonko et al., 2009a, b). However, these multi-drug resistance observed among some of the antibiotics used in this study, has now become a large and growing problem in infections that account for most of Africa's disease burden, including respiratory and diarrheal diseases (Okeke et al., 2007). Because antimi-

crobial resistance patterns are continually evolving and multi-drug resistant (MDR) organisms such as *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *S. aureus* and *Str. pyrogenes* undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles will continue to be essential to ensure the provision of safe and effective empiric therapies.

There are several limitations of this work. First, the inability to segregate nosocomial and community infections prevented a more detailed analysis of antimicrobial drug–resistance issues pertaining to community and hospital settings. Second, the use of different laboratory standards and methods potentially adds a degree of inaccuracy in the analyses. Third, routine laboratory data did not enable us to distinguish the different mechanisms of resistance, particularly among gram-negative bacteria, or to determine the presence of any predominant clone responsible for the high endemic levels of antimicrobial resistance. Nevertheless, the results can serve to direct any national effort aimed toward reducing the antimicrobial resistance problems of local hospitals. The reasons for the differences in antimicrobial drug–resistant patterns might be related to infection control practices or to timing of the introduction of resistant organisms. However, more research is needed to clarify these differences. We believe that our findings represent the endemic multi-drug resistant situation in our hospitals in Nigeria.

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