Incidence of Multi-Drug Resistance (MDR) Organisms in Abeokuta, Southwestern Nigeria

Iheanyi Omezuruike Okonko, Femi Adebiyi Soleye, Timothy A. Amusan, Ayoteju A. Ogun, Tolulope A. Ogumnusi and Joan Ejembi

1Department of Virology, Faculty of Basic Medical Sciences, University of Ibadan College of Medicine, University College Hospital (UCH), Ibadan, University of Ibadan, Ibadan, Nigeria
WHO Regional Reference Polio Laboratory, WHO Collaborative Centre for Arbovirus Reference and Research, WHO National Reference Centre for Influenza. HIV Reference Laboratory
2Medical Laboratory Unit, Department of Health Services, University of Agriculture, Abeokuta, P.M.B. 2240, Abeokuta, Ogun State, Nigeria
3Department of Veterinary Biochemistry, College of Veterinary Medicine, University of Agriculture, Abeokuta, Ogun State, Nigeria
4Department of Epidemiology, Medical Statistics and Environmental Health, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Nigeria
5Department of Biological Sciences, Ajayi Crowther University, Oyo, Oyo State, Nigeria
6Department of Clinical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria

Abstract: This study reports the incidence of multi-drug resistant organisms from clinical samples in Abeokuta, the capital city of Ogun State, located in the forest zone of southwestern Nigeria. Clinical samples which include: mid-stream urine, high vaginal swabs (HVS), sputum, ear swab and urethra swab were collected from 70(100.0%) patients; 40(57.1%) males and 30(42.9%) females. A total of 15 bacterial agents were obtained of which Klebsiella pneumoniae [9(37.0%)] was the highest. It was predominant in urine, HVS and sputum samples. This was followed by Escherichia coli [2(2%)] and Pseudomonas aeruginosa [2(13.0%)]. E. coli was only isolated from urine samples while P. aeruginosa was isolated from urethra swab. Staphylococcus aureus and Streptococcus pyrogenes had one strain [1(5.6%)] each isolated. S. aureus and Str. pyrogenes were only isolated from ear swabs. The study indicates a high prevalence of bacterial pathogens 54.0% and parasitic worms and protozoa. From the antibiogram, all bacterial isolates were sensitive to Ciprofloxacin and Ofloxacin and showed high resistance to Ampicillin, Chloramphenicol and Tetracycline. The Gram positive bacteria were also sensitive to Peflacin and Nitrofurantoin and showed high resistance to Gentamicin and Streptomycin. The Gram negative bacteria were highly resistant to Cotrimoxazole, Gentamicin and Streptomycin except one strain of P. aeruginosa from urethra swab and urine which were sensitive to Gentamicin and Streptomycin respectively. S. aureus Str. pyrogenes were sensitive to Ofoxacin, Ciprofloxacin and Peflacin and resistant to Ampicillin, Nitrofurantoin, Cotrimoxazole and Tetracycline. Str. pyrogenes were sensitive to Ofoxacin, Ciprofloxacin, Peflacin and Nitrofurantoin and resistant to Ampicillin, Cotrimoxazole, Tetracycline and Clafotab. Variations in susceptibility of the antibiotics to isolates in the clinical samples are discussed in relation to abuse of use. Antimicrobial drug resistance is a major problem in Nigeria. This study shows that a good percentage of people were infested by multi-drug resistance bacterial agents. The information provided in this study may be useful in improving control programmes directed against infectious diseases in the tropics.

Key words: Antibiogram studies • Antibiotics susceptibility testing • Incidence • Multi-drug resistance • Bacteria pathogens • Clinical samples
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 [1]. Over the last 60 years, bacteria and, in particular, those pathogenic for humans have evolved toward antimicrobial drug resistance. This evolution has 2 key steps: emergence and dissemination of resistance [2]. The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes [3]. Bacterial antimicrobial drug resistance is a worldwide problem that is exacerbated by the diminishing number of new antimicrobial drugs in the pharmaceutical pipeline [4-5]. This is an emerging public health problem, especially in hospitals of the newly industrialized countries of Asia and the Pacific [6]. For example, in the United States in 2002, resistance to ampicillin and ciprofloxacin among 5,192 Escherichia coli blood isolates was 47.8% and 13.3%, respectively [7]. During the past decade there has been a marked increase in resistance of bacteria to antimicrobial agents. 

Microorganisms have developed the ability to make altered receptors for antimicrobial agents, have prevented agents from reaching their receptors within the bacterial cell, now have enzymes to destroy antibiotics and have resistant metabolic pathways [8]. 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 [8]. Available therapeutic options for antibiotic-resistant organisms are severely limited, as these organisms frequently display a multidrug-resistant (MDR) phenotype [3, 9-10].

The prevalent organisms that are usually isolated from clinical samples such as urine are Escherichia coli, Staphylococcus aureus, Klebsiella spp., Pseudomonas aeruginosa, Proteus spp. Streptococcus faecalis and Enterobacter spp. The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment. Gram-negative bacteria have been found most frequently in UTIs cases by several authors with E. coli and Klebsiella spp. being the most predominant organisms [11-12]. Other bacterial pathogens frequently isolated include Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus faecalis [12]. Stewart et al. [13] recently reported the isolation of an unusual multiple resistant Corynebacterium from the urine of a comatose patient. The pathogen was reported to be resistant to sulphur-furazole, trimethoprin, nalidixic acid, cefazolin, floxacin, ofloxacin, norfloxacin, vancomycin and fusidin [12]. These prevalent microorganisms have been found to be resistant to most chemotherapeutic agents. In 2001, the World Health Organization (WHO) launched the first global strategy to counter this phenomenon [14], a key component of which is the development of surveillance programs to monitor trends in antimicrobial drug resistance and use [6,14].

E. coli is one of the main causes of both nosocomial and community-acquired infections in humans [15] and one of the microorganisms most frequently isolated from blood [7,16-18]. Pathogenic isolates of E. coli have a relatively large potential for developing resistance [7, 15,17,19]. In recent years, fluoroquinolone resistance has increased in some countries [7]. CTX-M-type extended-spectrum $\beta$-lactamase (ESBL) dissemination has been described [20-21] and reports of multidrug resistance are not infrequent [1, 19, 22]. Bacteria of the Klebsiella genus may cause numerous infections in human, which are often treated with beta-lactam antibiotics. Because of resistance of many Klebsiella spp. strains to beta-lactams, alternative antibiotic therapy can make use of aminoglycosides and quinolones [23]. The prevalence of strains resistant to selected aminoglycosides (gentamycin, amikacin, netilmicin) and quinolones (ciprofloxacin, norfloxacin, nalidixic acid) in the particular years was analyzed by Sekowska et al. [23] and a statistically significant increase of multidrug-resistant K. pneumoniae strains, was demonstrated in the analyzed material [23].

Pseudomonas 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, maybe of importance in cross-infection. P. aeruginosa infections of the lower respiratory tract can range in severity from colonisation (without an immunological response) to a severe necrotising bronchopneumonia [24]. Severe infections, such as pneumonia or bacteraemia, are associated with high mortality rates and are often difficult to treat, as the repertoire of useful anti-pseudomonal agents is limited (some beta-lactams, fluoroquinolones and aminoglycosides and the polymyxins as last-resort

Drugs); moreover, P. aeruginosa exhibits remarkable ability to acquire resistance to these agents [25]. Acquired resistance arises by mutation or acquisition of exogenous resistance determinants and can be mediated by several mechanisms (degrading enzymes, reduced permeability, active efflux and target modification). Overall, resistance rates are on the increase and may be different in different settings, so that surveillance of P. aeruginosa susceptibility is essential for the definition of empirical regimens. Multidrug resistance is frequent and clinical isolates resistant to virtually all anti-pseudomonal agents are increasingly being reported [25].

However, for S. aureus and Pseudomonas aeruginosa, an increase of resistance has been reported. The underlying mechanisms seem to be unchanged. On the other hand, multi-drug resistant Gram-positive bacteria including methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), penicillin-resistant Streptococcus pneumoniae (PRSP) and vancomycin-resistant enterococci (VRE) have been a serious problem in the medical community [26]. These problems of multi-drug resistance have been the driving force for the development of newer quinolones. The next generation of quinolone antibacterial agents will be potent against multi-drug resistant bacteria, such as MRSA and provide a lower rate of emergence in resistance. Evolution of bacteria towards resistance to antimicrobial drugs, including multidrug resistance, is unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable [2]. Therefore, the only means of dealing with this situation is to delay the emergence and subsequent dissemination of resistant bacteria or resistance genes. Resistance to antimicrobial drugs in bacteria can result from mutations in housekeeping structural or regulatory genes. Alternatively, resistance can result from the horizontal acquisition of foreign genetic information. The 2 phenomena are not mutually exclusive and can be associated in the emergence and more efficient spread of resistance [2].

The World Health Organization (WHO), the European Commission and the U.S. Centers for Disease Control and Prevention (CDC) have recognized the importance of studying the emergence and determinants of resistance as well as the need for control strategies [1]. Overarching surveillance programs monitoring antimicrobial drug-resistance trends on a national or regional level are present in Australia [6, 27] and Europe [6, 28]. Such is not the case in Singapore [6] and Nigeria, where surveillance efforts have generally been conducted only at the institutional level, with limited sharing and analysis of data [6]. As a result, the actual scale of local antimicrobial drug resistance is not well defined. Attempting to predict the future of the relationship between antimicrobial drugs and bacteria is conceptually challenging and potentially useful [2]. According to Courvalin [2], we have been aware for a long period that "everything that exists in the universe is the result of chance and necessity" (Democritus, 460-370 BC), which holds true for antimicrobial drug resistance. Most unfortunately and for various reasons, it is extremely difficult to think like a bacterium. In other words, predicting the emergence of resistance to a drug class by a precise molecular mechanism is nearly impossible (e.g., glycopeptide resistance in enterococci or plasmid-mediated resistance to fluoroquinolones) [2]. Among all the conceivable mechanisms of resistance, one cannot anticipate which will emerge first under natural conditions [29]. However, based on the understanding during recent decades of the physiology (genetics and biochemistry) of bacterial resistance to antimicrobial drugs, impressive progress has been made in the techniques for in vitro detection and for elucidation of resistance. This progress should, in turn, be helpful in delaying the second step of resistance: dissemination [2].

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures and preventing the spread of antimicrobial-resistant microorganisms [1,30]. 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 [3,31-33]. Therefore, this current study reports on the incidence of multi-drug resistant organisms from clinical samples in Abeokuta, the capital city of Ogun State, located in the forest zone of southwestern Nigeria in order to evaluate the antibiogram (antibiotic susceptibility pattern) of the bacteria isolated from these clinical samples. It also establishes the extent of multi-drug resistance of these pathogens to these drugs in the community under study.

**MATERIALS AND METHODS**

**Study Population:** Clinical samples were collected from a total of 70 patients attending Health Services Department of the University of Agriculture in Abeokuta, the capital city of Ogun State, located in the forest zone of...
Sample Collection: Clinical samples which include: mid-stream urine, high vaginal swabs (HVS), sputum, ear swab and urethra swab were collected from 70 patients. The samples were collected and labeled medical laboratory unit of the health services department of the University. These samples were analyzed within 30 minutes to 1 hour of collection.

Culturing, Characterization and Identification of Bacteria from Urine Samples: The samples were streaked on nutrient agar, Mac Conkey agar, Blood agar and eosin methylene blue (EMB) agar. The plates were incubated at 37°C for 24 h as described by Cheesbrough [34], where every specimen that yielded a pure heavy growth of bacterial pathogens were included in this study [12]. Isolates obtained after incubation were subcultured using isolation media. The pure isolates of bacterial pathogens were transferred to Nutrient agar slant and stored in the refrigerator at 4±1°C. Suspected bacterial species were characterized and identified according to standard bacteriological methods, Gram Stains and biochemical tests such as catalase, coagulase, indole production, citrate utilization, triple iron sugar utilization and methyl red-Voges Proskauer as highlighted by Cheesbrough [34].

Antibiotic Susceptibility Test: The antibiotic susceptibility patterns of the isolates to common antibiotics used in the hospital were determined using the agar-disk diffusion method on Mueller-Hinton agar described by Ebie et al. [11] and in the Manual of Antimicrobial Susceptibility Testing [35]. Five discrete colonies were inoculated into 5ml of sterile nutrient broth and incubated at 37°C over night. The broth culture was then diluted 1:10 with a freshly prepared nutrient broth to give a count of approximately 10^6 colonies per millimeter. An overnight broth culture of each isolate was uniformly spread onto the surface of the Mueller-Hinton plates. 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 and incubated at 37°C for 18 h. 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 degree of susceptibility of the test isolate to each antibiotic was interpreted as either sensitive (S) or resistant (R) by measuring the zone diameter of inhibition. The Gram positive antibiotic discs contained the following antibiotic concentrations: Nitrofurantoin (50mcg), Co-trimoxazole (25mcg), Peflacin 10mcg, Ciproflox 10 mcg, Ampicillin (25mcg), Gentamycin 10 mcg, Streptomycin 30 mcg, Ceporex 10 mcg, Tetracycline (25mcg), Septrin 30 mcg and Ampicillin 30 mcg; whereas the Gram positive antibiotic discs had the following concentrations: Ciprofloxacin 10 mcg, Ofloxacin 10 mcg, Gentamycin 10 mcg, Streptomycin 30 mcg and Chloramphenicol (20mcg). Interpretation of results was done using the zone of inhibition sizes. Zones of inhibition of ≥ 18mm were considered sensitive, 13-17mm intermediate and < 13mm resistant.

RESULTS

Table 1 shows the frequency of isolation of bacterial pathogens in clinical samples under study. Of the 70 (100.0%) clinical samples studied, 15 (21.4%) were positive for microscopy and cultures as shown in Table 1. The overall prevalence of these organisms in the clinical samples was 21.4% and females had higher overall prevalence of 12.9% than the males 8.6%, however, there was significant difference in the sex of the subjects studied (P=0.05) as shown in Table 1.

The distribution of resistant organisms in relation to sex is shown in Table 2. Incidence density and distribution of various antimicrobial drug-resistant bacteria isolated in clinical samples from Abeokuta, Nigeria from sexes is shown in Table 2. A total of 15 bacterial isolates comprising 2 Gram positive and 13 Gram negative bacteria were obtained. It was observed that 40.0% of the isolates were from the urine samples while 33.4%, 13.3% and 13.3% were from the HVS, ear swabs and urethra swabs respectively while neither bacterial isolates nor parasitic worms and protozoa was found in sputum samples (Table 2). Of the bacterial pathogens obtained, Klebsiella pneumoniae [8(53.3%)] were most predominant and this was followed by Pseudomonas aeruginosa [3(20.0%)], Escherichia coli [2(13.3%)], Staphylococcus aureus [1(6.7%)] and Streptococcus pyogenes [1(6.7%)]. K. aerogenes was the most common bacterium from this urine samples followed by E. coli which was found in equal number in both males and females while P. aeruginosa was the least isolate from urine. HVS samples had the highest occurrence of the K. pneumoniae obtained and were mostly isolated from females. E. coli was only isolated...
Table 1: Overall Incidence of Multi-Drug Resistant Pathogens in Clinical Samples in Abeokuta, Nigeria

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. Tested</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>40 (57.1)</td>
<td>6 (8.6)</td>
<td>34 (48.6)</td>
</tr>
<tr>
<td>Females</td>
<td>30 (42.9)</td>
<td>9 (12.9)</td>
<td>21 (30.0)</td>
</tr>
<tr>
<td>Total</td>
<td>70 (100.0)</td>
<td>15 (21.4)</td>
<td>55 (78.6)</td>
</tr>
</tbody>
</table>

Table 2: Distribution of resistant organisms in relation to sex

<table>
<thead>
<tr>
<th>Resistant Organisms</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bacterial Isolates</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>3 (20.0)</td>
</tr>
<tr>
<td>E. coli</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Str. Pyrogenes</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (100.0)</td>
</tr>
</tbody>
</table>

Table 3: Antibiogram of various antimicrobial drug-resistant bacteria

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Amp</th>
<th>Chlor</th>
<th>Cipro</th>
<th>Cotrim</th>
<th>Gent</th>
<th>Nitrof</th>
<th>Oflox</th>
<th>Pefla</th>
<th>Strepto</th>
<th>Tetra</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>E. coli</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>S. aureus</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>S. pyrogenes</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Key: Amp, Ampicillin; Pef, Peflacin; Cip, Ciprofloxacin; Gen, Gentamicin; Str, Streptomycin; Nit, Nitrofurantoin; Chl, Chloramphenicol; Cot, Cotrimoxazole, Ofl, Ofloxacin, Tet, Tetracycline; R, resistant; S, sensitive. Isolate origin: e ear Swab, h HVS, u Urine and ur Urethra swab

Table 4: Number and Percentage (%) Susceptibility and Resistance of various resistant bacteria

<table>
<thead>
<tr>
<th>Antibiotics (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp</td>
</tr>
<tr>
<td>Bacteria</td>
</tr>
<tr>
<td>K. pneumoniae</td>
</tr>
<tr>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>E. coli</td>
</tr>
<tr>
<td>S. aureus</td>
</tr>
<tr>
<td>S. pyrogenes</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Key: Amp, Ampicillin; Pef, Peflacin; Cip, Ciprofloxacin; Gen, Gentamicin; Str, Streptomycin; Nit, Nitrofurantoin; Chl, Chloramphenicol; Cot, Cotrimoxazole, Ofl, Ofloxacin, Tet, Tetracycline; R, resistant; S, sensitive. Isolate origin: e ear Swab, h HVS, u Urine and ur Urethra swab
from urine. *S. aureus* and *St. pyogenes* were the only isolates from ear swabs which were found only males and females respectively. *P. aeruginosa* was the only isolate from urethra swabs and was found only in males (Table 2).

The antibiograms of various antimicrobial drug-resistant bacteria are shown in Tables 3. The susceptibility patterns obtained revealed varying degrees of resistance and sensitivity to the antibiotics used in the screening. All bacterial isolates were highly resistant to Ampicillin, Chloramphenicol and Tetracycline. All Gram negative isolates were more resistant to Cotrimoxazole, Gentamycin, Streptomycin and Nitrofuratoin. All Gram positive isolates were also resistant to Gentamycin, Streptomycin, Cotrimoxazole and Nitrofurantoin. All bacterial isolates were highly sensitive to Ciprofloxacin and Ofloxacin. This was closely followed by Peflacin and Nitrofurantoin respectively. Gram positive isolates were sensitive to Nitrofurantoin and Cotrimoxazole. On the other hand, the Gram negative isolates were sensitive to Nitrofurantoin, Gentamycin and Streptomycin as shown in Table 3.

Table 4 shows the number and percentage susceptibility and resistance of the various resistance organisms. The result indicates that all bacterial isolates were highly resistant to Ampicillin (100.0%), Chloramphenicol (100.0%) and Tetracyline (100.0%). All Gram negative isolates were more resistant to Cotrimoxazole (100.0%), Gentamycin (92.3%), Streptomycin (92.3%) and Nitrofurantoin (69.2%). All Gram positive isolates were also resistant to Gentamyacin (100.0%), Streptomycin (100.0%), Cotrimoxazole (50.0%) and Nitrofurantoin (50.0%). All bacterial isolates were highly sensitive to Ciprofloxacin (100.0%) and Ofloxacin (100%). This was closely followed by Peflacin (53.3%) and Nitrofurantoin (33.3%) respectively. Gram positive isolates were sensitive to Nitrofurantoin (50.0%) and Cotrimoxazole (50.0%). On the other hand, the Gram negative isolates were sensitive to Nitrofurantoin (30.1%), Gentamycin (7.7%) and Streptomycin (7.7%) as shown in Table 4.

**DISCUSSION**

The findings of this current study showed that *Klebsiella pneumoniae* (53.3%) predominated over *E. coli* (13.3%) and *Staphylococcus* spp. This result compares favourably with the findings of Omonigho et al. [12] who found *Klebsisella* spp. to be more prevalent than *E. coli* in UTIs. This result is contrary to the findings of other workers who found *E. coli* more predominant over *Klebsisella* spp. in studies on UTIs [11-12, 36] and Chikere et al. [3] who reported *Staphylococcus epidermidis* (22) to be predominant pathogen over *S. aureus* (16), *Streptococcus* spp. (5), *E. coli* (4) and *K. pneumonia* (3) and 2 strains of *Proteus* spp., *En. aerogenes* and *B. cereus* in similar studies on clinical samples. Other pathogens isolated in order of prevalence include *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* but the 53.3% recorded for *Klebsisella* species however brings to light the fact that *Klebsisella* spp. are achieving more prominence as aetiologic agents of UTI than previously demonstrated by most workers [11, 37-39].

The most common pathogens isolated in these clinical samples were *Klebsiella* spp. (53.3%), *Pseudomonas aeruginosa* (20.0%), *Escherichia coli* (13.3%), *Staphylococcus aureus* (6.7%) and *Streptococcus pyogenes* (6.7%). This finding deviate from other reports which indicate that Gram-negative bacteria, particularly *E. coli* is the most implicating pathogen isolated in patients clinical samples from patients with UTIs [40-41] and other reports which also indicate the Gram positive bacteria, particularly *Staphylococcus epidermidis* to be the most common and predominant pathogen isolated in other clinical samples [3].

Antibiotics sensitivity tests revealed that all the bacterial pathogens isolated were susceptible to Ciprofloxacin and Ofloxacin in equal magnitude. They were also sensitive to some other antibiotics with *Escherichia coli* having the highest degree of sensitivity to Nitrofurantoin (100.0%) as opposed to Aiyegoro et al. [41] who reported a reduced sensitivity of *E. coli* to nitrofuratoin was observed in this study as only 63.6% of the *E. coli* was sensitive to the antibiotics. The sensitivities to Peflacin, Nitrofurantoin, Gentamicin, Streptomycin and Cotrimoxazole were 55.3%, 33.3%, 6.7% and 6.7% respectively. This is similar to the reports of Onifade et al. [40] who reported sensitivity to ofloxacin to be the highest (71.3%), Aiyegoro et al. [41] who also reported 97.1% sensitivity to Ofloxacin, 77.8% to Ciproflaxin and 50% to Nitrofurantoin. This is a deviation from the report of Chikere et al. [3] who reported the sensitivities of Gram negative isolates to Peflacin, Gentamycin, Streptomycin and Ciproflox to be 100%, 100, 90.9 and 63.6% respectively and Gram positive isolates to be 93.3 and 82.2% sensitivity to Gentamycin and Streptomycin respectively. Antibiotics such as Ciprofloxacin, Ofloxacin and Nitrofurantoin may be recommended for the treatment of urinary tract infection when treatment is necessary.
The antibiogram also revealed that all isolates were resistant to Ampicillin, Chloramphenicol and Tetracycline in equal magnitude as opposed to that reported by Aiyegoro et al. [41] who reported the sensitivity of *E. coli* to tetracycline to be 73.7%. Oteo et al. [1] reported that antimicrobial resistance, particularly to fluoroquinolones and third-generation cephalosporins, was increasing in *E. coli*. Resistance to Cotrimoxazole and Gentamicin in this study was quite high compared to those commonly reported. In this study, all the Gram negative isolates were resistant to Cotrimoxazole, Gentamicin Peflacin and Streptomycin. Out of the total Gram negative isolates that were also resistant to Peflacin and Streptomycin, *E. coli* were predominant. Also, in this study, 93.3% of the pathogens were resistance to cotrimoxazole. Resistance of *E. coli* to cotrimoxazole was 100% and is in contrast to results obtained elsewhere. A similar situation was described previously with sulfonamide resistance in the United Kingdom [42]. Cotrimoxazole resistance remained stable, approximately 30% in a study by Oteo et al. [1] and similar to the 27% reported by Alos et al. [43] in urinary tract infection isolates in Spain in 1993. Aiyegoro et al. [41] reported that 66.7% of the pathogens were resistance to Cotrimoxazole and that resistance of *E. coli* to cotrimoxazole was 57.9%.

A resistance of 100% was seen against Ampicillin, Chloramphenicol, Cotrimoxazole, Gentamicin, Streptomycin, Tetracycline and 75% were resistant to Nitrofurantoin, 50% to tetracycline but all were sensitive to ofloxacin. This result is similar to that reported by Aiyegoro et al. [41] who also reported that *Klebsiella* spp. showed a resistance of 66.7% against amoxicillin and cotrimoxazole, 55.6% to tetracycline and nitrofurantoin and that all were sensitive to ofloxacin. This is also similar to the study on the outbreak of multi-resistance *Klebsiella* in a neonatal intensive care unit in a hospital in Israel in which the *Klebsiella* isolates were resistant to chloramphenicol, gentamycin, cefuroxin but sensitive to quinolones [41].

The Gram negative isolates were mostly resistant to Ampicillin, Chloramphenicol and Tetracycline followed by Cotrimoxazole, Gentamicin, Nitrofurantoin and Streptomycin. Antimicrobial drug resistance in the Enterobacteriaceae (K. pneumoniae, E. coli) was prevalent for these antibiotics, *E. coli* accounted for the highest resistance to the above antibiotics. This agrees favourably with the findings of Chikere et al. [3]. It is also well documented that Gram negative bacilli harbour series of antibiotic resistant genes which can be transferred to other bacteria horizontally [44-47]. All the Gram negative bacilli isolated in this study namely *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have been shown to cause different nosocomial infections by other researchers [3, 9-10, 46-49].

Lastly among the Gram negative isolates, *P. aeruginosa* isolated in this study was 100% resistant to five out of the ten antibiotics *in vitro* (Ampicillin, Chloramphenicol, Cotrimoxazole, Nitrofuratoin and Tetracycline), 66.7% to Gentamicin and Streptomycin and 33.3% to Peflacin, but 100% sensitive to Ofloxacin and Ciprofloxacin and 66.7% sensitive to Peflacin. Multi-resistance *P. aeruginosa* was also isolated by Olouw and Oyetunji [50] in their study of nosocomial urinary tract infection and Fagade et al. [51] in their study on clinical samples. Aiyegoro et al. [41] also isolated multi-resistance *P. aeruginosa* in their study to determine the incidence of urinary tract infection in children and adolescents in Ile-Ife.

All Gram positive isolates in addition to Ampicillin, Chloramphenicol and Tetracycline, were also resistant to Gentamicin and Streptomycin. *Staphylococcus aureus* were predominant. Outbreaks of *S. aureus* resistant to β-lactam antibiotics have been frequently associated with devastating nosocomial infections [3, 45, 52]. In this investigation, *S. aureus* showed marked resistance to Ampiclox which is a β-lactam antibiotic. *S. epidermidis* were more resistant to Ciprofloxacin, Erythromycin, Norfloxacin and Floxapen. *S. epidermidis* is a major cause of nosocomial infections as well because of its ability to form biofilms on the surface of medical devices. According to Cloete [53], Villain-Guillot et al. [54] and Chikere et al. [1] bacterial biofilms are inherently resistant to antibiotics and host defenses and this could explain the reason for the high resistance seen in the strains isolated. *Streptococcus pyogenes* showed varying degrees of resistance (4 to 6 of the antibiotics) used in this study. This was also reported by Chikere et al. [1].

The antibiotic sensitivity test of this study shows that Ofloxacin was the most effective antibiotic in *in-vitro* testing followed by Ciprofloxacin which was effective against 100% of the pathogens. Similar results with quinolones have been reported by other authors. Aiyegoro et al. [41] reported 77.8% in their study. This low resistance of pathogens might be attributed to the fact that quinolones are relatively new antibiotics and have not been extensively used to warrant resistance developing against them by pathogens. This finding is in contrast with what was reported by Hsu et al. [6], who reported that K. pneumoniae and E. coli to be resistant to ciprofloxacin in their study on antimicrobial Drug
Resistance in Singapore Hospitals. Hsu et al. [6] also reported that Methicillin-resistant S. aureus (MRSA) strains showed correspondingly high resistance levels to ciprofloxacin (93.9%).

Antimicrobial resistance in this study, principally to Cotrimoxazole, Gentamicin Peflacin and Streptomycin, varied between the sexes, with isolates from female patients more resistant than those from male patients. This is in contrast to the trends that have been described recently in the United States [19] and the Netherlands [55] and in Spain [1] in which antimicrobial resistance, principally to ciprofloxacin and gentamicin, varied between the sexes, with isolates from male patients more resistant than those from female patients. Also in this study, antimicrobial drug resistance was generally more prevalent in HVS samples, but there was marked inter-clinical samples variation in resistance percentages. The HVS, urine and urethra swabs samples had high rates of antimicrobial drug resistance, whereas the ear swabs sample had much lower rates. A comparison between organisms isolated from HVS cultures and other cultures demonstrated statistically significant differences with regard to percentage resistance for S. aureus, Str. Pyrogenes, P. aeruginosa and the Enterobacteriaceae. The reason for these finding is not evident. In general, approximately 33.3% of all resistant organisms were isolated from HVS cultures.

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 [3, 56]. 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 [3, 31].

Prompt therapeutic intervention is therefore advocated in this current study as it is essential to prevent cases of asymptomatic infection from becoming symptomatic with resultant damage. This current study has shown beyond reasonable doubt that 21.4% of all the patients attending health services department of the University of Agriculture in Abeokuta, Southwestern Nigeria have pathogens present in their clinical samples. Therefore, the findings of this current study have in no doubt helped to ascertain the facts that the findings of previous works on detection and incidence bacterial pathogens and eggs/cysts of parasitic worms and protozoa are relatively similar. Though there could be slight variations but this could only be attributed to environmental factors, methods of samples collection and cultural methods.

It is apparent from the results of the antibiogram that the hospital investigated could be a potential reservoir of nosocomial pathogens. The high incidence of antibiotic resistant strains isolated from clinical samples of patients in this health services department, is worrisome and as such this issue needs to be addressed properly. We highly recommend the development of infection control programmes for the surveillance of nosocomial infections and epidemiologic typing of clinical isolates in hospitals in order to check the emergence and spread of antibiotic resistant infections in patients. The use of molecular biology techniques would also enhance the molecular identification of resistance genes [3, 57-58].

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.

In conclusion, this study determined the incidence of multi-drug resistant organisms in clinical samples from Abeokuta, the capital city of Ogun State, located in the forest zone of southwestern Nigeria. It also establishes the parasitic worms and protozoa load in the study population. Use of both incidence density and percentage resistance enabled a more nuanced analysis of the scale of the problem [6]. The pattern of isolates reported in this study is consistent with the usually reported pattern, with Klebsiella spp. being the most common organism isolated in cases of urinary tract infection. This was followed by P. aeruginosa, E. coli, S. aureus and S. pyrogenes. The study reveals the prevalence of multi-drug-resistance Klebsiella spp., P. aeruginosa, E. coli, S. aureus and S. 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 Ampicillin, Chloramphenicol and Tetracycline in equal magnitude as all the isolates were resistant to them and more than 60% of the isolates were also resistant to Cotrimoxazole, Gentamicin, Nitrofurantoin and Streptomycin in vitro and, as such, these antimicrobials may not be suitable for treating case of nosocomial or community acquired infection in this locality. However, all the isolates were sensitive to ofloxacin and ciprofloxacin and large proportion of the isolates were sensitive to Peflacin, Nitrofurantoin and should be considered as first line drugs for treating cases of nosocomial or community acquired infection in this environment. Ciproflaxin and ofloxacin are however best avoided in children as they have been shown to cause arthropathy in animal’s studies [41].

In this study, multi-drug resistance was frequent (50.0%) and increased by 75% during the study period (2002-2007). Multi-drug resistance in the United States among 38,835 urinary tract infection isolates was 7.1% in 2000 [19]. Such multi-drug resistance has important implications for the empiric therapy of infections caused by Klebsiella spp., P. aeruginosa, E. coli, S. aureus and S. pyogenes and for the possible co-selection of antimicrobial resistance mediated by multi-drug resistance plasmids [1, 22].

These results are not surprising. Previous institutional and local studies had already hinted at the extent of the problem in Nigeria [3, 40-41, 50]. Similar data have also been reported from other countries in the Australia [6, 27], Europe [6, 28], Asia Pacific region and Singapore [6, 27, 59-61]. In comparison with similar data from Europe [6, 28] and Australia [6, 27], prevalence of resistance in gram-negative organisms is much higher. 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 antimicrobial drug resistance situation in our hospitals in Nigeria. In view of the grave consequences of drug resistant organisms in patients, there is need for urgent action to control the situation. It is therefore recommended that routine microscopy, cultures and antibiotic sensitivity test of clinical samples of patients be carried out so as enhance in the administration of drugs for the treatment and management of clinical infections/diseases such as UTIs, ear infections, skin infections, nosocomial infections, respiratory tract infections etc. There should also be mass education and public awareness programmes on the importance of proper personal hygiene and good environmental sanitation habits.

Because antimicrobial resistance patterns are continually evolving and resistant organisms such as E. coli invasive isolates, Klebsiella spp., P. aeruginosa, S. aureus and S. pyogenes 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 [1]. Moreover, results obtained from this study must be used to implement prevention programs and policy decisions to prevent emergence and spread of antimicrobial resistance [1]. According to Hsu et al. [6], continued surveillance will also serve as an impartial feedback on the efforts of infection control programs for the future. Such surveillance of clinical microbiology isolates is a critical first step toward controlling the growing worldwide threat of antimicrobial drug resistance and WHONET is a useful tool in this respect [6]. More so, further extensive work should be done to ascertain the extent of these consequences of drug resistant pathogens in our environment.

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. Physicians worldwide have been encouraged to join public health authorities, the infection-control community and the pharmaceutical industry to curb the inappropriate use of antibiotics and promote responsible prescribing [62]. This will greatly help to improve all steps towards the prevention and control of drug resistant organisms in our community. Thereby enhance government efforts towards improving public health care system and providing “health for all” in Nigeria.

REFERENCES


