Antibiotic Resistance Profiles of Bacterial Pathogens from Private Hospitals in Dar Es Salaam, Tanzania

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Abstract

Objective: This study aimed to determine the prevalence rates of antibiotic resistance among pathogenic bacteria to widely used antibiotics.

Methods: Bacterial pathogens isolated from clinical specimens were identified by microbiological conventional methods (biochemical and colony morphology) then subjected to antimicrobial sensitivity testing against the antibiotics using Kirby-Bauer disk-diffusion method against 9 widely used antibiotics viz. Chloramphenicol (C30), Amoxicillin (AX25), Gentamicin (CN10), Cefuroxime (CMX30), Ciprofloxacin (CIP5), Ampicillin (AMC30), Doxycycline (DOC30), Ceftriaxone (CR030) and Ampicillin (AM10).

Results: A total of 304 bacterial isolates of were tested, of those 204(67.1%) were from mid-stream urine. The most frequently isolated bacteria were Escherichia coli (57.2%; n=174), trailed by Staphylococcus aureus (15.4%; n=47) and only 9 (3.0%) Proteus spp. Overall, 45.5% bacterial isolates were resistant to at least two of the antibiotics. About 60.0% (n=182) of pathogenic bacteria were susceptible to CIP5 and only 15.5% (n=47) isolates were sensitive to AM10, 16.4% (n=50) to DOC30 and 17.8% (n=54) to AX25.

Conclusion: The clinical isolates exhibited high prevalence rates of antibiotic resistance ranging from 60-70% to AM10, AX25 and AMC30 in this order. The observed high prevalence rate of antibiotic resistance emphasizes the need for routine antibiotic susceptibility testing and surveillance to avoid treatment failure and spread of antibiotic resistance.

Keywords: Antibiotic resistance, Pathogenic bacteria, Susceptibility patterns.

Introduction

Antibiotics/antimicrobial agents have substantially reduced the threat posed by microbial infectious diseases since their discovery in 1920s. The use of antibiotics has led to dramatic drop in deaths from diseases that were previously fatal [1]. However future effectiveness of antibiotics is somewhat in doubt because of emerging resistant microorganisms. The emergence of antibiotic resistance is further complicated by the fact that bacteria and their resistance genes are travelling faster and further [2-4].

Bacteria may be intrinsically resistant to more than one class of antimicrobial agents, or may acquire resistance by de novo mutation or via the acquisition of resistance genes from other microorganisms [5, 6]. There are several mechanisms through which bacteria can exert antibiotic resistance: by alteration of some cellular components in such a way that antibiotic does not interact with its receptor within or on the bacterial cell surface, and by enzymatic modification of the antibiotic affecting its configuration and rendering it ineffective [7]. These two resistance mechanisms are chiefly under environmental influences, which oblige bacteria to find better means of surviving in harsh conditions that create selective pressure; the survivors become resistant to the stressors (antibiotics) [8-10]. Airlines now carry more than two billion passengers annually, vastly increasing the opportunities for rapid and worldwide spread of infectious agents, including antibiotic resistant bacteria [11]. The spread of resistance is also facilitated by worldwide distribution of food [12], poor hygiene in health facilities as well as in the community among vulnerable populations particularly in resource-limited countries [13-15]. Easy availability of antibiotics as over the counter drugs and purchase without prescription on the internet or over-prescription of antibiotics, patients not completing antibiotic therapy and use of substandard antibiotics (having lower concentration of active ingredient) have contributed significantly to antibiotic resistance [13, 14].

In Tanzania, irrational use of antibiotics and their easy availability without prescription, use of Pharmaceuticals of doubtful quality and the HIV epidemic to a great extent have attributed to the development and spread of antibiotic resistance [13, 16]. Furthermore, some livestock keepers, and particularly poultry rearers use antibiotics intended for humans to treat their flocks; without veterinary doctors’ advices as means of cutting production costs [12, 17, 18].

Not only antibiotic resistance impedes provision of effective treatment but also increases morbidity and mortality [19-21]. The surge in antibiotic resistance observed in many low-income countries is potentially disastrous because of the lack of resources for purchasing more efficacious antibiotics that are always more expensive [22-24]. Therefore, this study sheds light on the current situation of antibiotic resistance in Tanzania which will also serve as caution to health care providers to take note on the routine empirical antibiotic treatment.

Materials and Methods

Study design and area

This was a prospective cross sectional study that involved collection of clinical specimens from three major private hospitals in Dar es Salaam namely Regency Medical Centre, Masana and Hindu Mandal Hospitals during a 4 month period. All clinically significant bacterial isolates were subjected to standard microbiological identification tests based on colony growth characteristic morphologies and biochemical tests for buttressing their identity [25]. These included isolates from mid-stream urine, feces, blood, pus, high vaginal secretion (HVS) and other body fluids. The collected isolates were transported to the Pharmaceutical Microbiology Laboratory at Muhimbili University of Health and Allied Sciences (MUHAS) using routine transport medium (Pepitone water-Oxoid, UK). Two strains of reference bacteria from the American Type Culture Collection (ATCC) namely Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) that are conserved in the Laboratory of Microbiology/Immunology-School of Medicine (MUHAS) were employed as control bacteria. Each microorganism was sensitized by sub-culturing into freshly prepared Mueller-Hinton broth (Roth, Germany) followed by an overnight incubation at 37°C. Then turbidity of each bacterial suspension was compared to that of McFarland 0.5 standard turbidity (equivalent to 1.5 x 10^8 cfu/ml), prior to performing antibiotic susceptibility profiling as per Clinical Laboratory and Standards Institute (CLSI) guidelines [26].
Antibiotic susceptibility testing

Each identified bacterial isolate was subjected to antibiotic susceptibility analysis against 9 widely used antibiotic viz. CN10 (10µg), Ceftriaxone (30µg), AM10 (10µg), and C30 (30µg); Oxoid, United Kingdom; and CIP5 (5µg), AX25 (25µg), Doxycline (30µg) and CXM30 (30µg) as well as AMC30 (30µg); Bioanalyse, Turkey. All assays were performed in Mueller-Hinton agar plates (Roth, Germany) using the Kirby-Bauer disk-diffusion method. Following an overnight incubation at 37°C, diameters of inhibition zones (IZ) were determined in millimeter.

Interpretation of antibiotic susceptibility profiles

Confirmation of antibiotic resistance involved determination of IZ for each bacterium that were compared to those of reference strains of bacteria and categorized as susceptible (S), intermediate (I) or resistant (R) in accordance with CLSI standards [26].

Statistical data analysis

All above procedures were aseptically conducted in triplicate and repeated for statistical purpose and consistency of results. Therefore, the IZ were expressed as mean. Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS 17.0, software (SPSS Inc., Chicago, IL). Analysis of variance (ANOVA) for differences in means of IZ among clinical isolates and in respecto reference strains of bacteria for each antibiotic and difference were considered significant when p<0.05.

Ethical aspects

Ethical clearance was sought from MUHAS Research and Ethical Committee. Likewise, permission to conduct the study was obtained from respective hospital authorities. No direct contacts with patients were made since the bacterial isolates were obtained from “leftover specimens”. These were specimens that had been already utilized for diagnostic purpose but properly handled and kept as they were pre-requested for research purpose. However, patients’ names from which specimens were obtained were not disclosed and only serial numbers were used for specimens processing.

RESULTS

A total of 304 clinical bacterial isolates were subjected to antibiotic susceptibility tests. Majority 67.1% (n=204) of the bacterial isolates were from mid-stream urine, of those E. coli constituted 57.2% (n=174). The least number of bacteria, each with total of 3 isolates (1.0%) were obtained from blood, HSV and catheter sip specimens. Escherichia coli comprised of 57.2% (n=174) of the tested bacteria while minority (3.0%; n=9) were Proteus spp (Table 1).

Table 1: Sources of clinical isolates

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Sources</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECO</td>
<td>Urine</td>
<td>158 (52.0)</td>
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<tr>
<td>STA</td>
<td>Sputum</td>
<td>18 (5.9)</td>
</tr>
<tr>
<td>PRO</td>
<td>-</td>
<td>10 (3.3)</td>
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<tr>
<td>PSE</td>
<td>-</td>
<td>6 (2.0)</td>
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<tr>
<td>SAL</td>
<td>-</td>
<td>6 (2.0)</td>
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<tr>
<td>KLE</td>
<td>-</td>
<td>10 (3.3)</td>
</tr>
<tr>
<td>STR</td>
<td>-</td>
<td>6 (2.0)</td>
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<tr>
<td>Total</td>
<td>204 (67.1)</td>
<td>12 (3.9)</td>
</tr>
</tbody>
</table>

Key: ECO- E. coli; STA-Staphylococcus spp; PRO-Proteus spp; PSE-Pseudomonas spp; SAL-Salmonella spp; KLE-Klebsiella spp; STR-Streptococcus spp. (-) implies NIL

Majority (69.7%; n=212) of the tested bacterial isolates were resistant to AM10 followed by AMC30 184 (60.5%) and AX25 182 (59.9%) as shown in Table 2. Of 9 assayed antibiotics, AM10 was the least effective, exhibiting resistance to about 70% of the tested isolates. However Proteus spp. isolates were the most susceptible yielding mean IZ of 18.67±1.53 mm, while P. aeruginosa isolates were the least susceptible with mean IZ of 6.0±0.56 mm.

Table 2: Antibiotic resistance profiles of clinical isolates

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Number of tested bacterial isolates/Antibiotics</th>
<th>R</th>
<th>I</th>
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<td>ECO</td>
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Key: Ref: STA-control Staphylococcus; Ref-ECO-control E. coli; C30-Chloramphenicol (30µg); AX25-Amoxicillin (25µg); CN10-Gentamicin (10µg); CXM30-Ceftriaxone (30µg); CIP5-Ciprofloxacin (5µg); AM30-Amoxiclav (30µg); DO30-Doxycline (30µg); CR30-Ceftriaxone (30µg); AM10-Ampicillin (10µg).
On average, about 45.7% (n=139) bacterial isolates were resistant to at least two of the tested antibiotics while 33% (n=100) of the tested isolates were susceptible to the antibiotics. Majority (74.1%; n=129) of E. coli and 80% (n=8) Klebsiella isolates of were resistant to AM10. Moreover, susceptibilities to AM10 among the tested bacterial isolates differed significantly (p=0.004; df=8; x²=166.23) as depicted in Fig. 1-3.

About 58.5% (n=178) of the tested bacterial isolates were susceptible to CIP5 and CN10 while all of them were resistant to P. aeruginosa (100%; n=22) and about 86.4% (n=19) were sensitive to CRO30 as shown in Table 2). All Proteus isolates were resistant to C30 and produced the lowest IZ against the antibiotic with mean IZ=9.67±3.21 mm.

**Susceptibility profiles of S. aureus, E. coli and Streptococcus isolates**

When S. aureus, E. coli and Streptococcus isolates were compared to reference strains of bacteria, no significant differences in their susceptibility (IZ) to C30 (p=0.160; F=1.599; df=6). Were observed. However, S. aureus was the most sensitive to the antibiotic yielding a mean IZ=19.1±9.71 mm and the least was E. coli with IZ=15.9±8.93 mm. CIP5 exerted the largest IZ (the most effective) against the pathogenic bacteria; and no significant differences in susceptibilities were revealed among the 3 tested bacteria (p=0.055, F=2.434; df=4). Again S. aureus exhibiting the largest IZ=25.1±14.92 mm. Though apparently, the least effective antibiotic was CXM30; the Independent-T-Test revealed no significant differences in IZ with respect to their respective reference strains (p<0.05) as shown in Fig. 1.

More than 50% (n>100) of the E. coli isolates exhibited resistance to the CXM30, AMC30 and AM10 (Table 2). High variability in antibacterial effects (IZ) was revealed among Streptococcus isolates against CIP5 (mean IZ=21.4±15.4 mm).

**Susceptibility profiles of Salmonella typhi and Klebsiella isolates**

All isolates of Klebsiella species were resistant to AX25 producing a mean IZ 6±2.21 mm. About 50% (n=11) Salmonella isolates were resistant to D030 but sensitive to C30 (Table 2). Comparisons of susceptibilities among bacterial isolates against CN10 revealed no significant differences (p=0.159; x²=106.130), though Salmonella isolates were the most sensitive (mean IZ=22.12±3.97 mm) as shown in Fig. 2.

The observed differences of susceptibilities to CIP5 among the bacterial pathogens were statistically significant (p=0.008; x²=538.40; df=8). All Salmonella isolates were sensitive to CIP5 (100%; n=22) and about 86.4% (n=19) were sensitive to CRO30 as indicated in Table 2. About 66.6% of Klebsiella isolates were equally susceptible to CIP5 and CN10 while all of them were resistant to AX25 (Fig. 2). High variability in antibacterial effects (IZ) was revealed among Klebsiella isolates against CIP5 (mean IZ=30.0±17.24 mm) as shown in Fig. 2.

**Fig. 1:** Antibiotic susceptibility profiles of E. coli, Streptococcus and S. aureus isolates

**Fig. 2:** Antibiotic susceptibility profiles of Klebsiella and Salmonella isolates

**Susceptibility profiles of Pseudomonas aeruginosa and Proteus isolates**

Although few in number, but all tested isolates of Proteus (n=9) and P. aeruginosa (n=19) were resistant to C30 (Table 2). Of these two pathogenic bacteria, Proteus isolates were more sensitive to C30 (mean IZ=17.1±10.10 mm) as compared to those of Pseudomonas spp. Nevertheless, some isolates of Pseudomonas yielded relatively higher IZ than those of both Proteus isolates and the reference bacterial strains (Fig. 1 and 3).

**Fig. 3:** Antibiotic susceptibility profiles of Pseudomonas and Proteus isolates

Pseudomonas isolates exhibited high variability in IZ to all assayed antibiotics with exception of AM10 to which they were resistant. Proteus spp. (18.5±6.36 mm) being the most sensitive to AM10. Most of the Proteus spp. isolates were resistant D030 and C30 exhibiting mean IZ of 8.3±3.24 mm and 6.7±3.21 mm respectively (Fig. 3). Pseudomonas isolates were very sensitive to CIP5 producing mean IZ 40.5±10.61 mm. Moreover, no statistically significant differences of IZ (p=0.438; F=0.985; df=3) were evident between the two pathogenic bacteria and the reference bacterial strain (p=0.576).

Comparisons of antibiotic susceptibilities among Pseudomonas and Proteus isolates against CN10 revealed no significant differences (p=0.159; x²=106.130); although Proteus isolates were the most sensitive producing mean IZ=22.0±9.16 mm. Equally, no significant differences (p=0.076; df=8; x²=4.957) in antibiotic susceptibilities to CXM30 were observed among the tested clinical isolates. However, P. aeruginosa (mean IZ=19.2±7.70 mm) were apparently the most susceptible to CXM30 as shown in Fig. 3. Generally, no statistically significant differences (p=0.164; x²=5.4; df=54) were observed between the pathogenic bacteria and reference bacterial strains against AMC30 (Fig. 1-3).
DISCUSSION

In most of health facilities in Tanzania, medical investigations and antimicrobial susceptibility tests are hardly performed due to financial constraints which may lead to misdiagnosis and treatment failure. Infections caused by resistant microorganisms fail to respond to the antimicrobial therapy resulting into prolonged treatment/hospitalization and increases chance of transmitting resistant strains of microorganisms to health individuals thus increasing morbidity and mortality in the population at large [21, 22]. When antibacterial drugs are used irrationally, such as to treat infections caused by parasites (for example, malaria) or viruses (such as the common cold), they provide no benefit to the patient and create selective pressure with possible needless adverse effects/reactions for the patients [15, 27].

Prescribing practice in resource-limited countries is usually patient's demand-oriented, which means the patient is usually prescribed with what can afford. Studies from around the world have shown that between 40 and over 90% of antibiotic prescriptions are unnecessary [22]. In some developing countries, empirically Antibacterial drugs are commonly available from unsanctioned providers. Such providers often reach out to people with limited access to orthodox health care, and are commonly not trained to diagnose infections or prescribe correctly. Accredited drugs dispensing outlets (ADDO) in Tanzania, is the best example; antibiotics are dispensed by unqualified personnel, though initially the ADDO were not meant for such an important role [28].

The observed high prevalence rate of resistant bacterial isolates revealed in this study is presumably because this pathogenic bacteria were obtained from clinical specimens of patients, who were suffering from various diseases for unknown period of time. Hence, the patients might have been constantly pre-exposed to the antibiotics and/or to sub-therapeutic doses of the same as well. Supporting such observation, previous study reported higher antibiotic resistance rates among pathogenic bacteria isolated from in-patients than from the out-patients or general practice [29]. This suggests that not only some drug resistant pathogenic bacteria could have been contracted during hospitalization, but also due to exposure of the bacteria to several antibiotics that exerts selective pressure among the pathogens spilling to antibiotic resistance [30].

CXM30 and CRO30 exhibited substantial effectiveness against the tested isolates. However, these antibiotics are neither available nor unaffordable by the majority of citizens [24]. Moreover, most of S. aureus and Streptococcus isolates were resistant to AMC30 as is a drug of choice for beta-lactamase producing bacteria [21]. The beta-lactamase producing bacteria are usually resistant to beta-lactam group of antibiotics, Cephalosporins inclusive. Klebsiella spp., E. coli, and Pseudomonas species are beta-lactamase producers that had previously manifested to be resistant to cephaplosporins [31]. Results revealed that some of the tested isolates were as resistant to AMC30 as to individual beta-lactams (AM10, AX25, CXM30 and CRO30). In such instance, prescribers might be obliged to seek for more efficacious antibiotics that definitely are also more costly and probably unaffordable for the majority. Occasionally, in Tanzania, antibiotic therapy is not laboratory-individualized or even by laboratory-inferred. Such circumstances, coupled with the high proportion of life-threatening infections that require immediate treatment, prescribers opt for empirical treatment and therefore antibiotic resistance can only be detected by therapeutic failure [32].

Almost all infectious diseases of major public health importance have now become resistant to a certain extent to several first line antibiotics [31]. Consequently, people infected with antibiotic resistant bacterial strains are more likely to have longer hospital stays, require more efficacious antimicrobial agents [21]. Failure to procure more efficacious antibiotics that are often more expensive may result into untimely death. Four of the most widely used and affordable antibiotics exhibited high resistance rates; C30 (42%), DO30 (44%), AX25 (60%) and AM10 (70%). These antibiotics were not very effective against potentially pathogenic bacteria such as P. aeruginosa and S. aureus. Nevertheless, the observed antibiotic resistance rates were less than that previously reported from Palestine [32].

Gram-positive and Gram-negative bacteria are well renowned for their susceptibility to C30, though most strains of P. aeruginosa are not. This study revealed that though all Pseudomonas isolates were resistant to C30 as it was expected, high variability in antibiotic susceptibility among the isolates was noticeable. Furthermore, the present study showed relatively higher antibiotic susceptibility of P. aeruginosa isolates to C30 as compared to AM10, which may be an indication of a change in antibiotic resistance trends. The observed change or variability in antibiotic susceptibility could have been attributed to 'positive' mutations as result of withdrawal and/or reduced prescription and usage of C30 resulting into deletion of the resistant genes [33].

Additionally, our findings revealed 100% (n=19) susceptibility of P. aeruginosa isolates to CIPS as compared to 34% previously reported [20]. Needless to say, a small number of Pseudomonas isolates tested in our study could significantly influence the findings. Cephalosporins (CXM30 and CR030) are broad spectrum antibiotics that have demonstrated to be very effective against both non-β-lactamase-producing gram-negative and many β-lactamase-producing bacteria [31, 34], which is in concordance with our present findings, in contrast to what had been reported in Nigeria [35].

Approximately 20% of Klebsiella pneumoniae infections in intensive care units in the United States are susceptible to third-generation cephalosporins. Such resistance in K pneumoniae to third-generation cephalosporins is typically caused by the acquisition of plasmids containing genes that encode for extended-spectrum β-lactamases (ESBLs), and these plasmids often carry other resistance genes as well [4,5,9]. The extensive use of antibiotics in the community and hospital settings might have fueled this crisis [28].

The resistance rates in Pseudomonas and Streptococcus isolates against Cephalosporins had previously reported to range from 92-100% [36, 37], which are higher than our findings. On the contrary, our results show relatively higher rates of CIPS-resistant E. coli (23.3%) as compared to (10.4%) in 2000 [36]. Increasing CIPS (fluoroquinolone)-resistance in many pathogens causing healthcare-associated and community-acquired infections has raised concerns about the future usefulness of this potent and frequently prescribed class of antibiotic [37, 38]. It is important, therefore, that CIPS sparing antibiotics be used only when necessary, since the antibiotic seems to be even more efficacious than AMC30 in treatment of some notorious infections [39].

The present study reveals high variability in antibiotic susceptibility among the pathogenic bacteria. Not only this is an indication of change in antibiotic resistance patterns, but also calls for further studies to determine the current guidelines for empirical therapy regimens, which vary from one country to another, and help with the establishment of effective infection control measures [14, 28]. The increase in rates of antibiotic resistant pathogenic bacteria advocates for regular review of antimicrobial sensitivity profiles among bacteria of clinical significance in our health facilities.

CONCLUSION

An overall low rate (31%) of susceptibility to commonly used antibiotics was revealed among the tested pathogenic bacteria. The highest rates of antibiotic resistance were exhibited by beta-lactams namely AM10, AX25 and AMC30 in that order. Slightly over 50% and 58% of the tested isolates were susceptible to CIPS and CR030. This study further recommends that continued surveillance of changes in resistance patterns is of utmost importance if effective management of infectious diseases is to be guaranteed. For cost-effective treatment of bacterial infections, practitioners should prescribe antibiotics based on the locally exhibited and established susceptibility patterns, which may avoid unnecessary use of more expensive second or third line antibiotics. The study also stresses the need to monitor changes in the antibiotic profiles for detection of new resistance traits, hence avoiding treatment failure.
REFERENCES


