Significance of Antibiotics Resistance Amongst Clinical Bacterial Isolates in Lagos

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Summary

In vitro susceptibility of several strains of six different species of clinical facultative pathogens involved in nosocomial infections in our hospital was investigated by agarose diffusion, broth dilution and Chequerboard titration testing. With disc diffusion method all the test strains, except Streptococcus pyogenes, were resistant to penicillin. 46% of the Klebsiella aerogenes and 73% of the Pseudomonas strains were generally resistant to cephalaxine. The minimum inhibitory concentration (MIC) of the antibiotics correlated well with the results of the disc diffusion tests. Synergistic effects were demonstrated by various combinations of gentamicin, ampicillin, cinoxacin, cefotaxime and cephalaxine against resistant strains of S. aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella aerogenes. Against S. aureus the effect of gentamicin/clindamycin demonstrated indifference. The need for stringent caution is strongly advocated in the selection of combination therapy for serious infections caused by the hospital bacterial strains particularly in antecentralis. The clinical microbiologist should be consulted at all times during the process of selection of an appropriate combined therapy for expert guidance.

Key Words: Antibiotics, Nosocomial infection, Penicillin resistance, Antibiotic synergism, Facultative pathogens, In vitro susceptibility.

Résumé

La sensibilité in vitro de plusieurs souches de six espèces d'organismes facultatifs mêlés dans l'infection nosocomiale à notre hôpital a été étudiée par des analyses de diverses méthodes. Ce sont la diffusion en disque, dilution de la bouillon et titration au tableau carreaux.

Streptococcus pyogenes a été penicillino-résistant. 46% de Klebsiella aerogenes et 73% de la souche de Pseudomonas ont été résistants au céphalexine. La concentration minimale inhibitrice (CMI) de la tigécycline a été en bonne corrélation avec les résultats de la diffusion en disque.

Les effets synergistiques ont été démontrés par plusieurs combinaisons de gentamicine, ampicilline, cinoxacin, cefotaxime et céfalexine contre les souches de S. aureus, Pseudomonas aeruginosa, Escherichia coli et Klebsiella aerogenes qui ont été résistants. L'effet de la combinaison de gentamicine et clindamycine sur S. aureus a resté inchangé.

Des mesures d'urgence sont nécessaires et vivement recommandées dans la sélection de la combinaison thérapeutique pour des infections sévères.

Introduction

Bacterial infections remain significant causes of morbidity and mortality in the developing countries. Monitoring of the susceptibility pattern of hospital pathogens on a constant bases and watch for development of resistance is not only necessary but should be mandatory. The problems of bacterial resistance to antibiotics is increasing in the tropical countries just as it is also worldwide. Evidence shows that for nosocomial infections, mortality, likelihood of hospitalization and length of hospital stay are at least twice as great for patients infected with drug-resistant strains compared with those infected with sensitive strains. Therefore, antibiotic resistance is of considerable health problem and economic burden and also places serious restriction on the treatment options for a host of important bacterial infections.

An attempt to combat the problem of drug resistance in life-threatening condition and seriously ill patients has led to the concept of antibiotic combination therapy with the aim of producing synergistic bacterial effects. For instance, synergistic action is usually desirable for the treatment of bacterial endocarditis particularly those caused by the Enterococcus spp, peptostreptococci, bacterial infection in severely immunocompromised patients, mixed post-surgical infections and septic arthritis especially in patients with underlying malignancies.

In view of the current trend of increasing isolation of antibiotic resistant bacteria in hospital practice, the present study reports our findings on the antibiotic resistance pattern of several strains of six different bacterial species responsible for about 90% of the nosocomial infections in our hospital and the activities of antibiotic combinations on both sensitive and resistant isolates.

Materials and Methods

Bacterial strains:

The following local clinical isolates (c) were used for this study: pseudomonas aeruginosa (59 isolates), Klebsiella aerogenes (40), S. pyogenes (25), S. aureus (96), Enterococcus faecalis (21), Group A streptococci (47) and Escherichia coli (156). They were isolated from clinical specimens from patients seen and managed at the College of Medicine, University of Lagos, P.M.B. 12003 Lagos, Nigeria.
Antibiotic Resistance in the Lagos University Teaching Hospital (LUTH), and identified by the standard methods\(^{(10)}\), they were preserved on agar slants until used.

Also included in this study were the following reference strains which were used as controls: *S. aureus* (ATCC 29213), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853). Five multiply resistant isolates of *P. aeruginosa* (strain Cl66), *S. aureus* (c566), *E. faecalis* (c46), *K. aerogenes* (c550) and *E. coli* (c640) were selected for the checkerboard synergy tests.

**Media:**

The various media used for these tests were: Brain heart infusion broth (BHB), and Mueller-Hinton broth (MHB) and agar (Oxoid), DST agar (Oxoid Plain), and MacConkey agar (Oxoid).

**Antibiotics:**

The following antibiotic sensitivity discs were used: methicillin (5 ug), chloramphenicol (10 ug), penicillin (2 units), cotrimoxazole (25 ug), terracycline (10 ug), gentamicin (10 ug), colistin (10 ug), clindamycin (5 ug), erythromycin (10 ug), cotrimoxazol (30 ug), ampicillin (10 ug and 25 ug) and cefazolin (30 ug). The potency powders of the following antibiotics were used for the minimum inhibitory concentration (MIC) and synergy testing: gentamicin (LUTH Pharmacy), chloramphenicol (LUTH Pharmacy), cefoxitin (APP (MSD), Armawo-Obin, Lagos), ampicillin and colistin (LUTH Pharmacy), ceftriaxone (Roche, Ikeja, Lagos) and clindamycin (UpJohn, Agbara Estate, Nig.).

**Determination of Susceptibility to Antimicrobial Agents:**

The disc diffusion method: A standard method\(^{(10)}\) was used for the diffusion method of antimicrobial susceptibility testing. Culture appropriate concentration of test organisms (10\(^{6}\) cfu/ml) corresponding to 0.5 McFarland standard were streaked by Stokes method\(^{(10)}\) onto Mueller-Hinton agar (Oxoid) or DST agar using non-toxic swab (Sterilin, Middlesex) as spreader and a mechanical rotator (Mast Laboratory Ltd., Liverpool, England) for even concenme spread. Each test was controlled by the appropriate reference strains and result interpreted as discussed by Stokes and Ridgway\(^{(10)}\).

**Determination of Minimum Inhibitory Concentration (MIC):**

A modification of the method of Peersdorf and Sherris\(^{(10)}\) was used to determine the MICs of the antibiotics by microdilution broth procedure. Serial doubling dilutions of the antibiotics in appropriate sterile broth, were prepared to give final concentrations from 0.01 ug/ml to 256 ug/ml for each antibiotic. 0.1 ml of approximately 10\(^{5}\) cfu/ml of the strains was used as standard inoculum throughout the experiments. Each test was controlled by standard reference organisms as appropriate. The MIC endpoints were determined as the lowest antibiotic concentrations with no visible growth.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>ATCC25923 (1)</th>
<th>ATCC27853 (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. aureus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methicillin</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftaxime</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
| **Susceptibility Test:**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Percentage of Bacteria resistant to Gram-positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>96</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>96</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>96</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>96</td>
</tr>
</tbody>
</table>

**Checkerboard Synergy Tests:**

Previously described method\(^{(10)}\) was used for this test. For each resistant organism, one doubling dilution of each of the two antibiotic combinations to be tested in BHB were made at twice their required MIC in sterile test tubes arranged 8 x 8 in vertical and horizontal rows, so that the final concentrations reflected their respective MICs. These concentrations were adjusted for each antibiotic combination as appropriate. Each tube was then inoculated with 0.1 ml of approximately 10\(^5\) cfu/ml of test organism and the inhibitory activities of these concentrations for the organisms checked after incubation for 18 h at 37°C. The degree of combined effect was then read noting the tubes showing no growth.
Results

**Disc Agar Diffusion Test:**

The antibiotic resistance profiles of the test organisms by the disc diffusion tests is shown in Table 1. The data showed that 4 - 11% of the *S. aureus* isolates were resistant to six of the 14 antibiotics. They were however highly resistant to penicillin (96%), ampicillin (84%), streptomycin (96%) and cotrimoxazole (90%). Fifty percent, 50%, 46% and 46% were resistant to tetracycline, cefazidime, cefoxatin and chloramphenicol respectively. Only 4% were resistant to methicillin in this study. The *E. faecalis* isolates were more resistant than the *S. aureus* to gentamicin (73%), Cotrimoxazole (91%), Cefoxitin (47%), and Streptomycin (100%). 86 — 94% of *Strep. pyogenes* were resistant to the two aminoglycosides and 56 — 70% to the newer cephalosporins tested.

Of immense significance was the finding of high percentage of resistant isolates of *E. faecalis* (56%) and *E. Coli* (81 %) to ampicillin. In addition 69%, 77% and 86% of the *E. Coli* isolates were resistant to cotrimoxazole, tetracycline and streptomycin respectively.

**Minimum Inhibitory Concentration (MIC)**

Only seven antibiotics were tested due to problems beyond our control. Table 2 shows the MIC<sub>u</sub> and MIC<sub>50</sub> of the seven antibiotics tested against some selected pathogens. Overall, the MIC results matched the disc diffusion results.

**Table 2:** Minimum Inhibitory Concentrations (MIC) of seven selected antibiotics for the clinical bacterial isolates

<table>
<thead>
<tr>
<th>Organism (No.)</th>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;u&lt;/sub&gt; (µg/ml)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (50)</td>
<td>Ampicillin</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>Kleb. aerogenes</em> (44)</td>
<td>Ampicillin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Streptomycin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>Ps. aeruginosa</em> (59)</td>
<td>Ampicillin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>S. aureus</em> (64)</td>
<td>Ampicillin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>E. Faecalis</em> (22)</td>
<td>Ampicillin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
<tr>
<td><em>Strep. pyogenes</em> (47)</td>
<td>Ampicillin</td>
<td>12</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

Clindamycin was inactive against all the enterobacteriaceae tested with MICs of >256µg/ml for *Ps. aeruginosa*, *K. aerogenes*, and *E. coli* (both the wild strains and the reference strain, ATCC, 25922). Clindamycin in this study inhibited both *S. aureus* and *E. faecalis* at MIC values of <0.1 µg/ml. This obviously is a remarkable observation against the latter organism. The resistance of *E. faecalis* against ampicillin, cefoxitin and ceftriazone was confirmed by the MIC<sub>50</sub> values of 32 µg/ml each.

The MIC<sub>50</sub> values of >256µg/ml chloramphenicol for each of *E. coli*, *K. aerogenes* and *Ps. aeruginosa* were recorded as confirmatory of the disc diffusion tests. *K. aerogenes* also showed unusually high level resistance to cefoxitin with MIC<sub>50</sub> of 64 µg/ml.

**Synergy Tests**

As shown table 3, *In vitro* testing for synergy revealed that a combination of gentamicin and ceftriazone was synergistic against high level gentamicin resistant strains of *S. aureus*, *E. coli*, *K. aerogenes* and *E. faecalis*. So was the combination of gentamicin/ampicillin. Gentamicin/clindamycin were also synergistic against the same strains of gentamicin.
Antibiotic Resistance in Lagos

resistant *S. aureus* and *E. faecalis*. Combination of gentamicin/chloramphenicol demonstrated additive effect against gentamicin resistant *Staphylococcus aureus* and *Escherichia coli*. While that of cefotaxime/imipenem in combination showed indifference effect on all the organisms. The combination of cefotaxime/imipenem in combination against multiple resistant *E. coli* and *K. aerogenes* showed additive effect.

A synergistic effect was demonstrated by a combination of gentamicin/collistin, gentamicin/ceftriazone or cefotaxime against gentamicin resistant strains of *P. aeruginosa* (not shown in the table). Against gentamicin sensitive but chloramphenicol resistant *P. aeruginosa*, gentamicin/chloramphenicol combination was antagonistic. Similarly a combination of cefotaxime/ampicillin was antagonistic against chloramphenicol resistant *P. aeruginosa*.

**Discussion**

The immediate striking feature of the data generated in this study showed that the *E. faecalis* strains isolated from our hospital are resistant to ampicillin and cotrimoxazole with relatively worrying level of resistance to cefotaxime, ceftriazone and chloramphenicol. *K. aerogenes* isolates were also resistant to cefotaxime, ceftriazone and chloramphenicol. This is a remarkable pattern of resistance in both organisms because it is of great therapeutic significance. Ampicillin is a recommended drug of choice for the treatment of enterococcal infections; in combination with gentamicin it has been a standard regime for treating serious *E. coli* such as enterococcal endocarditis (43, 44). Reliable sensitivity of *E. faecalis* to ampicillin has been the general experience worldwide. A phenomenon of superinfection by the enterococci is commonly seen in clinical practice from some hospitals which has largely followed some third generation cephalosporin therapy or use of new B-lactam agents. Ceftriaxone and aztreonam, which has no activity against enterococci. Even though, *Enterococcus* species have always been regarded as maverick streptococci with their atypical behaviour including variable susceptibility to a number of antibiotics, ampicillin still remains the drug of choice. However, the vagaries seen in the susceptibility of the *E. faecalis* in this report may be explained, in the part, by the relatively few number may be explained, in the part, by the relatively few number of isolates tested, the abuse to which ampicillin usage has been subjected in our hospital and perhaps to alterations in the penicillin-binding proteins. In any case our experience is not an isolated one. This finding of ampicillin-resistant enterococci is indeed supported by the report of Spring beron whose entire 18 clinical isolates were resistant to every B-lactam antibiotics tested including ampicillin.

*K. aerogenes* resistant to cefotaxime and ceftriazone is surprising but also not peculiar to this study. In recent studies which compared our isolates with those from other reports, we reported a similarly high prevalence resistance rate to these agents among the Lagos isolates. This is also consistent with our previous experiences. The population of Lagos is presumed to be more affluent than the rest of the country and this might also be able to indulge in indiscriminate use of these novel expensive newer generation cephalosporins. This speculation, may be supported by the fact that resistance of this nature may arise from circumstances which determine whether microorganisms acquiring new resistance mechanisms achieve a selective advantage. Strains of *Klebsiella* spp. possesses a newly described inducible B-lactamase capable of rendering these antibiotics effective are increasingly being isolated from clinical specimens now-a-days.

Amoxicillin, included in this study, ceftriaxone has the highest in vitro activity against *Pseudomonas aeruginosa* strains. It inhibited all the gentamicin- and colistin-resistant strains. Therapeutically, this drug may indeed be superior to some aminoglycosides in the treatment of outbreak of pseudomonas infections. Experiences in the neonatal unit to combat outbreak of infection caused by resistant *P. aeruginosa* is in support of this assertion (Rotimi, V.O. and Adebiyade, A; personal observation). However, because of the tendency to induce B-lactamase in vivo, this drug should be reserved for the treatment of life-saving infections caused by *P. aeruginosa* and some serious gram-negative systemic infections. Its use as prophylactic agent or in empiric situation should be with extreme caution.

Penicillinase-producing strains of *S. aureus* are extremely common in Nigeria. Nearly 95% of our strains were resistant to penicillin and ampicillin, and with a relative increase resistance to the third generation cephalosporins. Ceftriaxone and cefotaxime, which have appreciable anti-staphylococcal activities. Similar findings have been documented in Italy, France, and England. However, experience with penicillin-resistant strains vary from hospital to hospital and it is useful for each hospital to determine its own prevalence rate. The isolation of chloramphenicol-resistant *S. aureus* remains high in Nigeria. Although not of great therapeutic significance, this finding suggests the greater use of chloramphenicol in treating many infections in the country. The sensitivity of our *Strep. pyogenes* strains remain consistent with our experience in the last 10 years. No change in sensitivity pattern has occurred. Penicillin still remains the drug of choice.
The combination of gentamicin plus colistin was synergistic for the colistin-resistant *P. aeruginosa* strains tested. Under some clinical circumstances it may be logical to use such a combination especially in immunocompromised patients in whom combination therapy may be necessary. Despite this clinical infection has become out-dated because of its poor in vivo action. Gentamicin/Cefoxitin combination was also synergistic against the multiple-resistant strains of *P. aeruginosa*. This observation is consistent with some earlier reports which demonstrated in vivo synergism between the β-lactams and the aminoglycosides (21, 22). Most importantly certain combinations were found to be antagonistic. Gentamicin combined with chloramphenicol produced antagonistic effect against gentamicin sensitive strains and cefoxitin/ampicillin combination was antagonistic on cefoxitin sensitive strains.

Clindamycin and cefoxitin combination was investigated because of their known individual efficacies in the treatment of mixed infections with aerobes and anaerobes. An additive effect was observed. This is an important finding which should inform the selection of this combination in seriously ill patients with polymicrobial infections. For this reason using this combination instead of single agents in mixed infections involving anaerobes may be prudent. Clindamycin, cefoxitin or metronidazole combined with an aminoglycoside would be a more appropriate therapeutic option in mixed infections.

Gentamicin combination with clindamycin or ampicillin produced synergistic effects on gentamicin resistant strains of *E. faecalis*, *E. coli*, *K. aerogenes* and *S. aureus*. This is particularly encouraging as resistance is synergy between gentamicin and ampicillin by high level gentamicin resistant *Enterococcus* species has been reported. Gentamicin/chloramphenicol combination showed indifferent activity to all the four species. This observation together with the antagonistic effects on *P. aeruginosa* make use of this combination therapy clinically unadvisable under any circumstances. Certainly not in any serious infection like meningitis or sepsisemia.

It is very important clinically to continue to monitor the development of resistant bacterial strains worldwide. The situation in Lagos and Ibadan gives cause for concern and does not appear to be abating as demonstrated in this study.

Acknowledgement

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References


