Evaluation of resistance pattern of the multi-drug resistant (MDR) bacteria isolated from burn wounds

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Out of 10 random burn wound swab samples, 15 isolates were found which included Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, Shigella spp. Pseudomonas aeruginosa, Citrobacter spp. and Escherichia coli. Antibiogram assay revealed that four of them were multi-drug resistant (MDR) strains, i.e, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and E. coli which were further selected for a comparative analysis of resistance through determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by using chloramphenicol and tetracycline. In case of tetracycline, the highest MIC value was estimated to be 30 µg/ml and the highest MBC value was found to be 60 µg/ml for the 4 MDR strains tested. Whereas, against chloramphenicol, the highest MIC value was 62.5 µg/ml and the highest MBC value was 125 µg/ml for all the MDR strains except for E. coli, which exhibited absolute resistance.

Key words: MDR; Chloramphenicol; Tetracycline; MIC; MBC

Burn wounds are extremely prone to infection as they present a suitable site for microbial proliferation. Infection is an important cause of mortality in burns. It has been estimated that 75% of all deaths following thermal injuries are related to infections (1). The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, immunocompromised status of the patients, invasive diagnostic and therapeutic procedures and prolonged ICU stay (2). Burn wounds can harbor more diverse groups of microbes than other wounds as they present already damaged cells with highly nutritious cell exudates. Clinical isolates are more prone to drug resistance than non-clinical isolates (3-5).

Chloramphenicol and tetracycline are considered as the prototypical broad-spectrum antibiotics. Chloramphenicol (45-60 mg chloramphenicol/kg body weight) (6) is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. Due to resistance and safety concerns, it is no longer a first-line agent for any indication in developed nations, although it is sometimes used topically for eye infections (7, 8). It is not active against Pseudomonas aeruginosa but remains the first choice of treatment for staphylococcal infections. However, use of chloramphenicol has been reported to associate some side effects including aplastic anemia, bone marrow suppression, gray baby syndrome and leukaemia (9-12). Nevertheless, clinical burn wound isolates have been found to be resistant against the standard doses of chloramphenicol (13). Besides the problems projecting through the use of chloramphenicol, the general usefulness of tetracycline has also been rendered ineffective in many cases mostly due to the drug resistance. Resistance of Pseudomonas aeruginosa against tetracycline has been significantly noted in burn patients (13, 14).

Based on these evidences, the present study was conducted to understand the efficacy of the two most common broad-spectrum antibiotics, chloramphenicol and tetracycline, against the emerging MDR clinical bacteria.

MATERIALS AND METHODS

10 samples were randomly collected from burn patients admitted in Dhaka Medical College Hospital (DMCH) Burn Unit using sterile cotton swabs. After a series of laboratory techniques including the examination of growth on Mannitol salt agar, Eosin-methylene blue, MacConkey agar, Xylose lysine deoxycholate agar and Cetrimide agar, 15 isolates were identified (including Bacillus cereus, Shigella spp., Citrobacter spp., Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and E. coli) and confirmed through morphological and biochemical tests. Applying the Kirby Bauer antibiotic disc diffusion method (15) against Penicillin (PG10), Gentamycin (GM10), Ampicillin (AP10), Chloramphenicol (C30), Nalidixic acid (NA30), Novobiocin (N030), Imipenem (IPM10), Ciprofloxacin (CIP5), Tetracycline (T30), Vancomycin (VA30), Mezlociline (MZ75) and Trimethoprim-sulphamethoxazole (SXT25), the most resistant strains (Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and E. coli) were selected for further study.

To prepare the stock solution of chloramphenicol, 10 mg of chloramphenicol powder was weighed and re-suspended in 10 ml of sterile distilled water resulting in the final concentration of 1 mg/ml chloramphenicol solution. For the working solution, 250 µl of the stock solution was added to 750 µl of sterile distilled water. This gave a working solution of 250 µg/ml chloramphenicol solution. A 4 times greater dilution was used for conducting the MIC and MBC (125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.625 µg/ml). An initial load of approximately 10⁶ cells (0.5 McFarland standard) were introduced in to each tube.

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For the tetracycline stock solution, 10 mg of sterile tetracycline powder was added to 10 ml of sterile distilled water to give a final concentration of 1 mg/ml. From this stock solution 60 µl was pipetted out to be added to 940 µl of sterile distilled water giving a final concentration of 60 µg/ml. For the MIC and MBC experiments, a 4 times two-fold dilution was used (30 µg/ml, 15 µg/ml, 7.5 µg/ml and 3.75 µg/ml). A load of approximately 10^5 cells (0.5 McFarland standard) was used in each tube.

**RESULTS**

After conducting the antibiotic sensitivity tests against PG10, GM10, AP10, C30, SXT25, NA30, NO30, CIP5, IPM10, T30, MZ75 and VA30, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E. coli* were found to be resistant against all the antibiotic discs except Imipenem (10 µg) and *Staphylococcus aureus* showed resistance against all antibiotics except Chloramphenicol (30 µg). Tetracycline (30 µg) and Imipenem (10 µg). *Bacillus cereus*, *Shigella* spp., *Citrobacter* spp. were found to be more susceptible than *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E. coli* (Table 1).

*S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *E. coli* were subjected to high concentrations of *E. coli* (Table 1).

From this stock solution 60 µl was pipetted out to be added to 940 µl of sterile distilled water to give a final concentration of 1 mg/ml. For the tetracycline stock solution, 10 mg of sterile tetracycline powder was added to 10 ml of sterile distilled water to give a final concentration of 1 mg/ml. From this stock solution 60 µl was pipetted out to be added to 940 µl of sterile distilled water giving a final concentration of 60 µg/ml. For the MIC and MBC experiments, a 4 times two-fold dilution was used (30 µg/ml, 15 µg/ml, 7.5 µg/ml and 3.75 µg/ml). A load of approximately 10^5 cells (0.5 McFarland standard) was used in each tube.

**DISCUSSION**

Current studies have left little doubt that popular antibiotics are becoming more and more unsuccessful due to the emergence of MDR bacterial strains. Interestingly the clinical samples have been proven to be more resistant than other samples.

**S. aureus**, **K. pneumoniae**, **P. aeruginosa** and **E. coli** were more resistant than other samples.

Intrigued by these facts, the current study was planned. The purpose was to carry out MIC and MBC for some commonly used popular antibiotics and evaluate their efficacy against clinical samples. Chloramphenicol is such a drug that has faced a fall in its popularity as a therapeutic agent. In consistence to the other studies, the present study also reveals that the clinical samples are more resistant to chloramphenicol (*Klebsiella pneumoniae* having an MBC of 125 µg/ml and *E. coli* showing absolute resistance). This may be as chloramphenicol causes a bacteriostatic effect by binding to the 50S ribosomal subunit and inhibiting the transpeptidation step in protein synthesis. Resistance may occur by any of three mechanisms, by reducing membrane permeability, 50S ribosomal subunit modification or enzymatically elaborating chloramphenicol acetyltransferase (16-19).

**TABLE 1.** Antibiogram of pathogenic bacterial isolates from burn wounds

<table>
<thead>
<tr>
<th>Organisms</th>
<th>PG 10</th>
<th>GM 10</th>
<th>AP 10</th>
<th>C 30</th>
<th>SXT 25</th>
<th>NA 30</th>
<th>NO 30</th>
<th>CIP 5</th>
<th>IPM 10</th>
<th>T 30</th>
<th>MZ 75</th>
<th>VA 30</th>
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<tbody>
<tr>
<td>S. aureus</td>
<td>R</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>
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<tr>
<td>Bacillus cereus</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
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<td>R</td>
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<tr>
<td>Shigella spp.</td>
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<td>S</td>
<td>S</td>
<td>S</td>
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<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
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<td>E. coli</td>
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<td>R</td>
<td>R</td>
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<td>R</td>
<td>S</td>
<td>R</td>
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<td>P. aeruginosa</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>S</td>
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<tr>
<td>Citrobacter spp.</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<td>S</td>
<td>R</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>K. pneumoniae</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
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</table>

PG10 = Penicillin G 10 µg; GM10 = Gentamicin 10 µg; AP10 = Ampicillin 10 µg; C30 = Chloramphenicol 30 µg; SXT25 = Trimethoprim-sulphamethoxazole 25 µg; NA30 = Nalidixic acid 30 µg; NO30 = Novobiocin 30 µg; CIP5 = Ciprofloxacin 5 µg; IPM10 = Imipenem 10 µg; T30 = Tetracycline 30 µg; MZ75 = Mezlocillin µg; VA30 = Vancomycin 30 µg

**TABLE 2.** MBC values against chloramphenicol

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Concentration of Chloramphenicol (µg/ml)</th>
<th>MBC value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250</td>
<td>125</td>
</tr>
<tr>
<td>S. aureus</td>
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<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumoniae</td>
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</table>
Resistance to chloramphenicol in *S. aureus* is most frequently due to the activity of an inducible detoxification enzyme, chloramphenicol acetyltransferase (20).

Tetracycline, though not as unsuccessful as chloramphenicol, has been ineffective in many clinical cases. In this study, *E. coli* and *Klebsiella pneumoniae* had an MBC of 60 µg/ml whereas tetracycline reaches a concentration of 5-12 µg/ml after a single dose of 250 mg or 500 mg dose (21).

Our recent study encourages herbal or natural remedies rather than traditional antibiotic drugs. *Aloe barbadensis*, used as a natural remedy for burn since ancient times, was proven as an answer to MDR bacterial threats (22).

In light of this current study, it is evident that traditional antibiotics used for therapeutic reasons are becoming more and more ineffective due to the rise of MDR bacteria or the so called “superbugs”. Studies should be conducted to find reliable and successful alternative medications for these MDR bacterial strains.

**ACKNOWLEDGEMENTS**

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**REFERENCES**