ORIGINAL ARTICLE

Stamford Journal of Microbiology, December 2013. Vol. 3, Issue 1, p. 6-8
ISSN: 2074-5346

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

Noshin Azra Rahman, Asma Akhter and Nusrat Jahan Urmi‡

Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka 1217, Bangladesh

Received 21 March 2013/Accepted 7 May 2013

Out of 10 random burn wound swab samples, 15 isolates were found which included *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, Shigella* spp. *Pseudmonas 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; Chloramohenicol; 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 are 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), Chloramohenicol (C30), Nalidixic acid (NA30), Novobiocin (NO30), Imipenem (IPM10), Ciprofloxacin (CIP5), Tetracycline (T30), Vancomycin (VA30), Mezlociline (MZ75) and Trimethoprim-sulphamethoxozole (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 two-fold 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 10^8 cells (0.5 McFarland standard) were introduced in to each tube.

‡Correspondingauthor. Mailing address: Nusrat Jahan Urmi, Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217, Bangladesh; E-mail: nusrat_mb@yahoo.com

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 10^8 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*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa* and *E. coli* (Table 1).

S. aureus, K. pneumoniae, P. aeruginosa and *E. coli* were subjected to high concentrations of chloramphenicol and tetracycline, two common broad spectrum antibiotics. The MIC for *S. aureus, K. pneumoniae*, and *P. aeruginosa* were 15.625 µg/ml, 62.5 µg/ml and 31.25 µg/ml respectively for chloramphenicol; and 15µg/ml, 30 µg/ml and 30 µg/ml, respectively for tetracycline. The MBC for *S. aureus, K. pneumoniae*, and *P. aeruginosa* were 31.25 µg/ml, 125 µg/ml and 62.5 µg/ml for chloramphenicol (Table 2) and 30 µg/ml, 60 µg/ml and 60 µg/ml for tetracycline (Table 3), respectively. Even though, *E. coli* was sensitive to high doses of tetracycline (MBC measuring 60 µg/ml), it showed absolute resistance to chloramphenicol.

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.

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/m l 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

Organisms	Antibiotic											
	PG 10	GM 10	AP 10	C 30	SXT 25	NA 30	NO 30	CIP 5	IPM 10	T 30	MZ 75	VA 30
S. aureus	R	R	R	S	R	R	R	R	S	S	R	R
Bacillus cereus	S	R	R	S	R	R	R	R	S	S	R	R
Shigella spp.	R	S	S	S	R	R	R	R	S	S	R	R
E. coli	R	R	R	R	R	R	R	R	S	R	R	R
P. aeruginosa	R	R	R	R	R	R	R	R	S	R	R	R
Citrobacter spp.	R	R	R	S	S	R	R	S	S	S	R	R
K. pneumoniae	R	R	R	R	R	R	R	R	S	R	R	R

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

TABLE 2. MBC values against chloramphenicol

Organisms	Concentration of Chloramphenicol (µg/ml)					MBC value
	250	125	62.5	31.25	15.125	
S. aureus	-	-	-	-	+	31.25 µg/ml
E. coli	+	+	+	+	+	Absolute resistance
P. aeruginosa	-	-	-	+	+	62.5 µg/ml
K. pneumoniae	-	-	+	+	+	125 µg/ml

TABLE 3. MBC values against tetracycline

Organisms	Concentration of Tetracycline (µg/ml)					MBC value
	60	30	15	7.5	3.75	
S. aureus	-	-	+	+	+	30 µg/ml
E. coli	-	+	+	+	+	60 µg/ml
P. aeruginosa	-	-	+	+	+	30 µg/ml
K.pneumoniae	-	+	+	+	+	60 µg/ml

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 bacteria (13). *Aloe barbadensis* was successful in preventing MDR bacterial growth in more effectively in comparison to traditional antibiotics (13). In recent years, a range of wound dressings with slow-release silver (Ag) compounds have been introduced, including Acticoat, Actisorb Silver, Silverlon, and others. They propose a better 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

Authors thank Stamford University Bangladesh for the technical facilities and financial support to carry out the experiments.

REFERENCES

1. **Vindenes, H., and R. Bjerknes.** 1995. Microbial colonization of large wounds. Burns **21** (8): 575-579.
2. **Pruitt, B. A., A. T. MacManus, S. H. Kim, and C. W. Goodwin.** 1997. Burn Wound infections: current status. World J. Surg. **22**: 135-145.
3. **Greenland, S.** 2001. Ecologic versus individual-level sources of bias in ecologica estimates of contextual health effects. Int. J. Epidemiol, **30**: 1343-1350.
4. **Koopman, J. S., and I. M. Longini.** 1994. The ecological effects of individual exposures and nonlinear disease dynamics in populations. Am. J. Public Health **84**: 836-842.
5. **Steinke, D., and P. Davey.** 2001. Association between antibiotic resistance and community prescribing: a critical review of bias and confounding in published studies. Clin. Infec. Dis. **33 (**Sppl 3): S193-S209.
6. **Arrioja-Dechert, A.** 2006. Compendium of veterinary products. North American Compendiums, Inc., Port Huron, MI.
7. **Falagas, M. E., A. P. Grammatikos, A. Michalopoulos.** 2008. Potential of old-generation antibiotics to address current need for new antibiotics. Expert Review of Anti Infective Therapy **6** (5): 593–600.
8. **Rose, C., et al.** 2002. Emergence of resistance of vancomycin-resistant *Enterococcus faecium* in a thermal injury patient treated with quinupristin-dalfopristin and cultured epithelial autografts for wound closure. Burns **28** (7): 696-8.
9. **Rich, M., R. Ritterhoff, and R. Hoffmann.** 1950. A fatal case of aplastic anemia following chloramphenicol (chloromycetin) therapy. Annals of Internal Medicine **33** (6): 1459–1467.
10. **Yunis, A. A.** 1989. Chloramphenicol toxicity: 25 years of research. Am. J. Med. **87** (3N): 44N–48N.
11. **McIntyre, J., and I. Choonara.** 2004. Drug toxicity in the neonate. Biology of the Neonate **86** (4): 218–221.
12. **Shu, X., et al.** 1987. Chloramphenicol use and childhood leukaemia in Shanghai. Lancet **2** (8565): 934–937.
13. **Rahman, N. A., and S. R. Rahman.** 2010. Multidrug-resistant (MDR) bacteria associated with burn wound infections and antimicrobial activity of leaf gel of *Aloe barbadensis* against some isolates. Bangladesh Journal of Medical Science **16** (1): 33-37.
14. **Ranjbar, R., et al.** 2011. Characterization of *Pseudomonas aeruginosa* strains isolated from burned patients hospitalized in a major burn center in Tehran, Iran. Acta MedicaIranica **49** (10): 675-679.
15. **Bauer, A. W., W. M. Kirby, J. C. Sheris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Pathol. **45**: 149-158
16. **Butaye, P., A. Cloeckaert, and S. Schwarz.** 2003. Mobile genes coding for efflux-mediated antimicrobial resistance in Gram- positive and Gram-negative bacteria. Int. J. Antimicrob. Agents **22**: 205-210.
17. **Paulsen, I. T., M. H. Brown, and R. A. Skurry.** 1996. Proton- dependent multidrug efflux systems. Microbiol. Rev. **60**: 575-608.
18. **Toro, C. S., et al.** 1990. Clinical isolates of a porin-less *Salmonella typhi* resistant to high levels of chloramphenicol. Antimicrob. Agents Chemother. **34**: 1715-1719.
19. **Ettayebi, M., S. M. Prasad, and E. A. Morgan.** 1985. Chloramphenicol-Erythromycin resistance mutations in a 23S rRNA gene of *Escherichia coli*. J. Bacteriol. **162**: 551-557.
20. **Murray, I., A. and W. V. Shaw.** 1997. O-acetyltransferases for chloramphenicol and other natural products. Antimicrob. Agents Chemother. **41** (1): 1-6.
21. **Gordon, J. M., et al.** 1981. Concentration of tetracycline in human gingival fluid after single doses. J. Clin. Periodontol. **8** (2):117-121.
22. **Silver, S., L. T. Phung, and G. Silver.** 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. Journal of Industrial Microbiology and Biotechnology **33** (7): 627-634.