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IN VITRO ACTIVITY OF FIVE ANTIBIOTICS AGAINST METHICILLIN AND GENTAMICJN RESISTANT *STAPHYLOCOCCUS A UREUS.*

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Summary

One hundred and seven strains of methicillin and gentamicin resistant ***Staphylococcus aureus*** were investigated for sensitivity to five other antimicrobial agents by the plate dilution method. 99% were inhibited by sodium fusidate at concentrations of 0.125mg/L and vancomycin at 2mg/L. Rifampicin at concentration of 0.015mg/L inhibited 98% of the strains and clindamycin at 0.03mg/L, inhibited 95%. 48% of strains were inhibited by amikacin at concentration of 2mg/L and 99% at 4mg/L.

INTRODUCTION

Staphylococcus aureus resistant to methicillin (MRSA) and methicillin and gentamicin (MGRSA) has emerged as an important cause of hospital-acquired infection in recent years. Its role in life threatening infections is well documented in current medical literature from many countries, including Malaysia[1]. At present, vancomycin is the recommended treatment for infections caused by MGRSA. However, other antibiotics have been suggested on the basis of ***in vitro*** sensitivity testing[2,3].

The isolation of MGRSA from clinical specimens and its incidence in a major Malaysian hospital have been reported[4,5]. We now present the ***in vitro*** activity of five antibiotics against these strains. It is hoped that these results will be helpful in the selection of antibiotics for the treatment of infections caused by MGRSA.

MATERIAL AND METHODS

One hundred and seven strains of MGRSA isolated from in-patients of the General Hospital, Kuala Lumpur were evaluated. The strains were isolated in the Diagnostic Microbiology Laboratory, Universiti Kebangsaan Malaysia from pus (51), body surface swabs (47), blood (4), pharyngeal specimens (3) and urine (2).

Routine antibiotic sensitivity testing

Sensitivity testing was carried out using the disc diffusion technique on Diagnostic Sensitivity Test Agar (Oxoid) by the Stokes' method. The discs evaluated were penicillin (10 units), erythromycin (15ug), clindamycin (10ug), cotrirnoxazole (trimethoprim 1.25ug, sulphamethoxazole 23.8ug), gentamicin (10ug), fucidin (10ug), vancomycin (30ug), rifampicin (30ug), amikacin (30ug) and methicillin (10ug). The plates were incubated for 18—24 hours at 37°C except for methicillin sensitivity testing for which plates were incubated at 30°C.

Minimum inhibitory concentrations

Minimum inhibitory concentrations (MIC) were determined by the agar dilution method, using a multi-point inoculator (Dynatech). The following antibiotics were evaluated: methicillin (Beecham), gentamicin (Roussel), sodium fusidate (Leo), rifampicin (Merrel), vancomycin (Eli Lilly), clindamycin (Upjohn) and amikacin (Bristol Meyers). An appropriate range of concentrations of each drug was prepared in sterile water and incorporated into known volumes of Diagnostic Sensitivity Test Agar (Oxoid), achieving the desired concentration. The inoculum was an overnight broth culture diluted to contain approximately 10^5 colony forming units per ml. The plates were incubated at 37°C, except for methicillin sensitivity testing, for which plates were incubated at 30°C and read after 24 and 48 hours[6].

Methicillin resistant strains were selected on the basis of disc diffusion test. The strains were uniformly resistant to gentamicin. Minimum inhibitory concentration towards methicillin and gentamicin was determined as described above. Strains with MIC of > 32 mg/L for methicillin and > 25 mg/L for gentamicin were designated as MGRSA and were selected for testing against other antibiotics.

RESULTS

In the disc diffusion test, the strains were sensitive to cotrimoxazole, sodium fusidate, vancomycin and rifampicin and resistant to tetracycline, penicillin, erythromycin, methicillin and gentamicin. The strains were sensitive to cotrimoxazole but dissociated resistance was observed in the presence of erythromycin. 90% of the strains were sensitive to amikacin.

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Table 1 shows the activity of 107 strains of MGRSA against amikacin, clindamycin, sodium fusidate, rifampicin and vancomycin determined by the agar dilution method. 99% were inhibited by sodium fusidate at 0.125 mg/L and vancomycin at 2 mg/L. Rifampicin at concentration of 0.015 mg/L inhibited 98% of the strains. Clindamycin was nearly as effective and 95% were inhibited at 0.03 mg/L. However, only 48% of strains were inhibited by amikacin at concentration of 2 mg/L, although at 4 mg/L, 99% were inhibited.

DISCUSSION

Sodium fusidate (fucidin), vancomycin, clindamycin and rifampicin were active against the 107 strains of MGRSA included in the present study. With one exception, the strains were inhibited by fucidin at a concentration of 0.125 ml/l. This differs from the experience of Moorhouse *et al.*, who have reported a high incidence of fucidin resistance in their strains[2]. Both fucidin and rifampicin should be administered with a second antibiotic to prevent the emergence of resistant mutants.

Rifampicin in combination with minocycline was effective in an *in vitro* study against MRSA, according to Yourassowsky *et al.*[3] Our strains were not tested against minocycline. Although they were sensitive to cotrimoxazole by the disc diffusion test, for septicaemic patients, vancomycin as a single agent or fucidin in combination with clindamycin can be recommended.

Gentamicin resistance is a common characteristic of methicillin resistant staphylococci and resistance towards other aminoglycosides may develop. Only 50% of the strains tested in this study were inhibited by amikacin at 2 mg/L and it should be prescribed with caution.

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TABLE 1

MINIMUM INHIBITORY CONCENTRATION OF 107 STRAINS OF GENTAMICIN AND METHICILLIN RESISTANT *STAPH AUREUS*

Concentration of antibiotics in mg/L	No. of strains inhibited by antibiotics at each concentration				
	Amikacin	Clindamycin	Sodium fusidate	Rifarnpicin	Vancomycin
⩾ 64		5			
32					
16					
8	6				
4	49		1		1
2	28				7
1	22				86
0.5	1				13
0.25					
0.125			75	1	
0.06			26		
0.03		102	5		
⩽ 0.015				105	

REFERENCES

1. Jamal F, Shong HK, Mahmooth T. Septicaemia caused by methicillin and gentamicin resistant *Staphylococcus aureus* in cases of burns. Jernal Perubatan 1983; 1: 53–8.
2. Moorhouse EC, Mulvihill TE, Jones L, Mooney D, Falkiner FR, Keane CT. The *in vitro* activity of some antimicrobial agents against methicillin resistant *Staphylococcus aureus.* J Antimicrobial Chemother 1985; 15: 291–5.
3. Yourassowsky E, Van Der Linden MP, Lismont MJ, Crokaert F. Combination of minocycline and rifampicin against methicillin and gentamicin resistant *Staphylococcus aureus.* J Clin Pathol 1981; 34: 559–63.
4. Jamal F, Antibiotic sensitivity pattern of *Staphylococcus aureus* and *Pseudornonas aeruginosa* in the General Hospital, Kuala Lumpur. Jernal Perubatan 1978; 1: 1–4.
5. Mohd Rohani Jais. Ujian *in vitro* aktiviti asid klavulanik amoxycillin dan augmentin terhadap strain-strain *Staphylococcus aureus* yang methicillin resistan. Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia 1982. 55 pp, dissertation.
6. Hewitt JH, Coe AW, Parker MT. The detection of methicillin resistance in *Staphylococcus aureus.* J Clin Pathol 1969; 26: 899–913.