Original Article

Antimicrobial susceptibility pattern of clinical isolates of Methicillin Resistant Staphylococcus aureus

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Abstract

Objective: To study the antimicrobial susceptibility pattern of MRSA isolates from patients in a tertiary care hospital.

Methods: This was in-vitro study on MRSA isolates received from clinical samples in the department of microbiology during one year (March 2004- February 2005).

All samples were processed by conventional method using sheep blood agar, MacConkey's agar and Chocolate agar plates. Staphylococci were identified by catalase, coagulase and D'nase tests. Antimicrobial susceptibility testing of all isolates was performed on Mueller-Hinton agar plates by modified Kirby Bauer's Disc Diffusion method. The disc used Oxacillin (1µg), Cephalexin (10µg), Ofloxacin(5µg), Fusidic acid (10µg), Penicillin (10µg), Vancomycin (30µg), Erythromycin (15µg), Gentamicin (10µg), Teicoplanin (30µg), Amikacin (30µg) and Clindamycin (2µg).

Results: Our results indicate that out of 190 positive isolates of S. aureus, 82 (43%) were found to be MRSA. These strains were also resistant to many other antistaphylococcal antibiotics.

Conclusion: A total of 82 (43%) MRSA were isolated from various clinical samples. Pattern of first line antistaphylococcal antibiotics is changing. Antimicrobial susceptibility testing is crucial in the treatment of these patients (JPMA 57:2;2007).

Introduction

Staphylococcus aureus (S. aureus) is a common human pathogen. Penicillin used to be the drug of choice to treat infections caused by S. aureus. Soon after the introduction of benzylpenicillin into clinical use in the 1940s, it became clear that some strains of S. aureus were resistant, due to production of ß-lactamase. Under the selective pressure of penicillin usage, the prevalence of penicillin resistant strains increased, particularly in hospitals. History repeated itself in early 1960s when the introduction of the ß-lactamase-stable, penicillin-derived methicillin was rapidly followed by the emergence of strains of methicillin- resistant S. aureus (MRSA).[1] In MRSA the mechanism of resistance does not involve inactivation of the antibiotic, but expression of a novel cell wall synthesizing enzyme (penicillin-binding protein 2) with low affinity for all ß-

lactams which is a product of mecA gene.[2]

Drug of choice to treat infections caused by MRSA is vancomycin. It is a glycopeptide and has been used to treat infections caused by MRSA all over the world.[3] Fusidic acid, one of the drugs which remains effective against MRSA, interferes with protein synthesis. Its principal activity is against staphylococci, including MRSA.[4]

Epidemiological studies suggest that hospitals of all sizes are facing the problem of MRSA. The problem appears to be increasing regardless of hospital size and control measures for MRSA. MRSA has become the commonest nosocomial infection throughout the world, causing a wide range of hospital infections.[5,6]

Antimicrobials are among the most valuable therapeutic agents in modern healthcare. Over the past six decades with the increasing use of antimicrobial agents for therapeutic purposes a variety of antimicrobial resistance has been observed.[7] With multiple antimicrobial agents and resistance mechanisms, selection of the appropriate antimicrobial agent for therapy of infection is challenging, both for empiric therapy and for infection with a defined pathogen. In-vitro antimicrobial susceptibility test results are important in guiding the choice of antimicrobial.

The rational of our study was to determine the antimicrobial susceptibility pattern of MRSA isolates from patients in a tertiary care hospital.

Material and Methods

The study was conducted over a period of one year (March 2004-February 2005) and included 190 strains of S. aureu, isolated from various clinical samples. Each sample was collected in a sterile container. The samples were cultured on sheep blood agar, MacConkey's agar and Chocolate blood agar plates. These plates were incubated at 37^{o}C for 24 hours, isolates of S. aureus were confirmed by gram staining, Catalase, Coagulase, Mannitol fermentation and D'nase test following standard microbiological procedures.[9]

Antibiotic susceptibility testing was done by Kirby Bauer's Disc diffusion technique, following NCCLS guidelines. Antibiotics used were Oxacillin (1µg), Cephalexin (10µg), Ofloxacin(5 µg), fusidic acid (10µg), Penicillin (10µg), Vancomycin (30µg), Erythromycin (15µg), Gentamicin (10µg), Teicoplanin (30µg), Amikacin (30µg), Clindamycin (2µg). ATCC S.aureus 25923 was used as a sensitive control strain.

Statistical analysis

The data was fed on computer package "EPI-info" 6.0 software of CDC (centre for disease control, Atlanta, USA). Test of proportion was applied to compare the resistance of antibiotics in MRSA and MSSA infections. Test of proportion was also applied to compare prevalence of MRSA in hospital and community.

Results

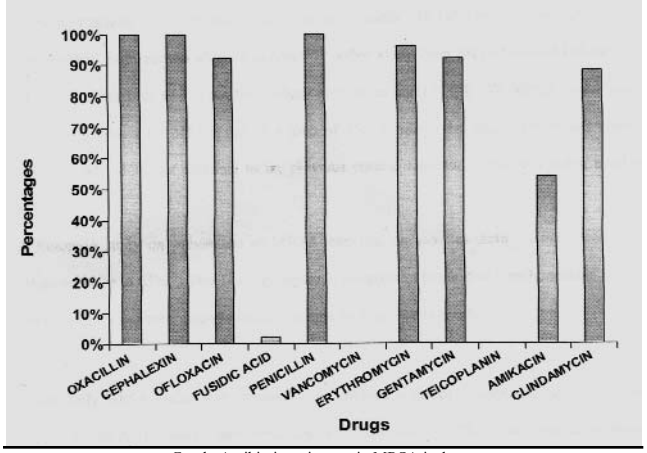
Table 1 shows out of 190 S. aureus, 82 (43%) isolates were MRSA. The sample were of pus, blood, bone, sputum, tracheal aspirate, urine, ear swab, conjuctival swab, wound swab, abscess, tissue, pleural fluid, nasal swab, umbilical sinus swab, pleural fluid, chest test tube and bronchial lavage collected from inpatient and outpatient departments of Dr. Ziauddin Hospital.

Table 1. Percentage of MRSA.

Source	Total Staphylococcus aureus	MRSA	%
Pus samples	113	37	32
Blood	15	8	53
Bone	2	1	50
Sputum	1	1	100
Tracheal Aspirate	15	12	80
Urine	4	2	50
Ear Swab	12	5	41
Conjunctival swab	2	2	100
Wound Swab	11	6	36
Abscess Swab	2	0	0
Tissue	4	1	25
Pleural fluid	1	1	100
Nasal Swab	1	1	100
Umblical sinus swab	3	0	0
Pleural fluid	2	2	100
Chest tip tube	1	1	100
Bronchial lavage	1	1	100
	190	82	43

Table 2. Antimicrobial drug resistance in methicillin-resistant staphylococcus aureus (MRSA) isolates and in methicillin-susceptible s. aureus (MSSA), 2004-2005.

Antimicrobial drug	MRSA isolates (n=82)	MSSA isolates (n=108)	p value
	No. resistant (%)	No. resistant (%)	
Oxacillin	100	0	0.001
Clindamycin	90	6	0.001
Erythromycin	95	12	0.001
Fusidic acid	2	0	-
Gentamicin	93	5	0.001
Penicillin	100	81	0.001
Amikacin	54	0	0.001
Ofloxacin	92	2	0.001
Cephalexin	100	5	0.001
Vancomycin	0	0	-
Teicoplanin	0	0	-



Graph. Antibiotic resistance in MRSA isolates.

Table 2 shows various antibiotics tested against MRSA and MSSA. Methicillin sensitive staphylococcus aureus were sensitive to most antibiotics tested, while MRSA were resistant to most antibiotics except vancomycin, teicoplanin, fusidic acid and amikacin.

Graph shows antibiotic resistance pattern of MRSA. The antistaphylococcal agents tested were oxacillin, cephalexin, ofloxacin, fusidic acid, penicillin, vancomycin, erythromycin, gentamicin, teicoplanin, amikacin and clindamycin.

Discussion

In the last two decades prevalence of MRSA has steadily increased all over the world[10], including Pakistan.[11-13] A study done at Sargodha[11] in 1999 showed that 23% MRSA were isolated from various clinical samples. Another study from Mayo Hospital Lahore[13] in 2001 revealed that out of 350 positive isolates of S. aureus 135 (38.5%) had MRSA. In our study 82 (43%) isolates were MRSA out of a total of 190 S. aureus isolates from various clinical samples. This does not correlate with the previous studies done and indicates a rising trend of MRSA.

An European study on prevalence of MRSA infection on samples from intensive care, estimated close to 65%.[14] This is a high figure in comparison to our data which included clinical samples from inpatients, outpatients, critical care units and other wards.

In our study MRSA strains were resistant to all antibiotics except Vancomycin and Teicoplanin (100% susceptible), Fusidic aid (96%) and Amikacin (46%). Multidrug -resistance makes treatment more difficult. This may be due to over the counter availability of antibiotics and self-medication in Pakistan[15] and inappropriate use of antibiotics all over the world.

Vancomycin is the universally accepted drug of choice. We found all MRSA isolates to be susceptible to vancomycin. Similar results have been quoted by other authors.[16,17]

Conclusion

This study concludes that the incidence of MRSA is rising and pattern of antibiotic susceptibility to first line antibiotics is changing. Antimicrobial susceptibility testing of all S. aureus isolates is crucial for treatment of MRSA.

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