



Original Research Article

Detection of ESBL, MBL and MRSA among Isolates of Chronic Osteomyelitis and their Antibiogram

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ABSTRACT

Chronic osteomyelitis is a common cause of morbidity in the developing countries. Resistant causative organisms are frequently isolated from clinical material. Hence, isolation of organisms and performance of susceptibility are critical in the selection of antimicrobial therapy which will help in the control of infection. 100 pus samples taken aseptically were cultured aerobically at 37°C for 18–24 hrs on Blood and MacConkey agar plates. Culture isolates were identified by a series of standard biochemical reactions. Antibiotic susceptibility was tested on Mueller Hinton agar by Kirby Bauer disc diffusion method. Extended spectrum beta – lactamases (ESBL) was detected by phenotypic confirmatory disc diffusion test, Metallo Beta Lactamase (MBL) by Imipenem EDTA combined disc diffusion test and Methicillin Resistant *Staphylococcus aureus* (MRSA) by using cefoxitin disc. *Staphylococcus aureus* was most commonly isolated (32.9%) amongst a wide range of organisms including gram negative bacilli and coagulase negative staphylococci. The prevalence of MRSA, ESBL and MBL was 40%, 68.9% and 18.9% respectively. Many isolates were found to be resistant to commonly used empirical anti-microbial regimens. The high prevalence of beta – lactamases and degree of resistance to commonly used anti-microbials supports the importance of culture reports which provides important information to guide clinician's choice of empirical antibiotics.

Keywords

Chronic osteomyelitis, ESBL, MBL, MRSA

Introduction

Osteomyelitis has long been one of the most difficult and challenging problem (Canale and James, 2008). Advances in the identification of infections and early diagnosis of osteomyelitis have lead to improved management of osteomyelitis (Kaur *et al.*, 2008). Nevertheless, osteomyelitis is still difficult to treat effectively (Canale and James, 2008). The most important risk factors of osteomyelitis are trauma (primarily open fractures and

sever soft tissues injury), vascular insufficiency, diabetes, elderly, children, obesity and surgical wound infections (Abid *et al.*, 2008). The still dominant role of *S. aureus* could be confirmed, but also the increasing number of gram-negative bacteria. The mixed infection is obviously determined by gram-negative bacteria with their marked resistance to antibiotics (Augsburg, 1981).

Beta – lactamases are the most evolving mechanism of antibiotic resistance among the family Enterobacteriaceae due to the selective pressure imposed by inappropriate use of third generation cephalosporins, most often encountered in ICU settings (Rudresh and Nagarathnamma, 2011). Three major groups of such enzymes are usually distinguished, class C cephalosporinases (AmpC), ESBLs and MBLs, are of great concern (Fam *et al.*, 2006). These enzymes hydrolyze the amide bond of the four membered characteristic beta - lactam ring, thus rendering the antimicrobial ineffective (Prashant Durvas Peshattiwar and Basavaraj Virupaksappa Peerapur, 2011). Carbapenems represented a great advance for the treatment of serious bacterial infections caused by beta-lactam resistant bacteria (Hodiwala *et al.*, 2013). But extensive and sometime unnecessary use of the carbapenems, poor sanitation and large population has facilitated the emergence of carbapenem resistant bacteria. Two types of carbapenem hydrolyzing enzymes are there, one is serine beta lactamase (having Serine at their active site) and other is Metallo Beta Lactamase (MBL), containing metal ion that works as a cofactor for enzyme's activity (Debasrita Chakraborty *et al.*, 2010).

Methicillin resistant *Staphylococcus aureus* (MRSA) is prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide (Habeeb Khadri and Mohammad Alzohairy, 2010). *Staphylococcus aureus* infections used to respond to β -lactam and related group of antibiotics but the emergence of MRSA has posed a serious therapeutic challenge.

Infected and colonized patients in hospitals mediate the dissemination of MRSA strains, and hospital staff is the main source of

transmission. MRSA strains are difficult to eradicate as they are multidrug-resistant leaving glycopeptides as the drugs of choice (Shilpa Arora *et al.*, 2010).

Because of the heterogeneity of disease severity, anatomic location, organism, and host, treatment of osteomyelitis is complex, and must be individualized (Joseph M. Fritz and Jay R. McDonald, 2008). Chronic osteomyelitis generally cannot be eradicated without surgical treatment. The goal of surgery is eradication of the infection by achieving a viable and vascular environment. Antibiotics alone rarely can eradicate the infection for numerous reasons (Canale and James, 2008). In selecting specific antibiotics for the treatment of osteomyelitis, the type of infection, current hospital sensitivity resistance patterns, and the risk of adverse reactions must be strongly appraised (Mader *et al.*, 1999).

Emergence of multidrug resistant organisms leading to treatment failure is of concern. Hence the present study was conducted to detect such resistant organisms and to know their susceptibility pattern.

Materials and Methods

100 pus swabs were taken from chronic osteomyelitis patients under strict aseptic conditions. Direct smear examination was done. The samples were inoculated onto blood and MacConkey agar plates and incubated aerobically at 37°C for 18–24 hrs. The isolates were identified by standard procedures (Collee *et al.*, 2007).

Antibiotic sensitivity was done on Mueller Hinton agar by Kirby Bauer disc diffusion method using Clinical and Laboratory Standard Institute guidelines (CLSI, 2011).

Antibiotic discs used were: Ampicillin

(10µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25µg /23.75µg), Oxacillin (1µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Erythromycin (5µg), Clindamycin (2µg), Linezolid (30µg), Vancomycin (30µg).

Detection of ESBL

Phenotypic confirmatory test

Test organisms were inoculated into Mueller-Hinton agar as lawn culture. The ceftazidime (30 µg) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg discs) were placed. An increase of ≥ 5 mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer.

Detection of MBL:

Imipenem EDTA combined disc test

Two (10 µg) imipenem discs were placed on a plate inoculated with the test organism, and 10 µl of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of ≥ 7 mm was interpreted as a positive result for MBL production.

Detection of MRSA:

Methicillin resistance was detected by Cefoxitin disk diffusion test. Lawn culture was done onto Mueller–Hinton agar plate. A 30 µg cefoxitin disc was placed and incubated at 37°C for 24 hrs. The zone of inhibition of *S. aureus* 21 mm were considered as methicillin resistant.

Results and Discussion

Total 107 organisms were isolated from 100 samples, of which 49 were Gram positive and 58 were Gram negative. Of 49 Gram positive isolates, 35 were *S. aureus* and 14 were Coagulase negative *Staphylococcus*. *S. aureus* was the commonest organism isolated (32.9%). MRSA was detected in 14(40%) isolates of *S. aureus* and most of them were sensitive to vancomycin 14(100%), linezolid 14(100%), amikacin 11(78.5%) and cotrimoxazole 7(50.0%).

The prevalence of ESBL and MBL among 58 Gram negative isolates was 40(68.9%) and 11(18.9%) respectively. Most of the ESBL producers were sensitive to imipenem 33(82.5%), amikacin 21(52.5%) and ciprofloxacin 18(45.0%) and MBL producers to amikacin 5(45.4%).

The presence of wide spectrum of antibiotics has markedly decreased the morbidity and mortality caused by infections. But with a discovery of each new class of antibiotic, a new mechanism of resistance emerges and nullifies the effects of antibiotics. Among a variety of drug-resistance traits, ESBL and MBL producing gram negative bacilli with resistance to newer cephalosporins have been posing a significant challenge in clinical practice (Dinesh S. Chandel *et al.*, 2011).

Beta lactamases are often located on plasmids that are transferable from strain to strain and between bacterial species (Mark E. Rupp and Paul D. Fey, 2003). Therapeutic options for the infections which are caused by the beta lactamase producers have also become increasingly limited (Metri Basavaraj *et al.*, 2011). The prevalence of ESBL and MBL in our study was 68.9% and 18.9% respectively which is comparable with other studies (Fam *et al.*, 2006; Rajesh

Kondian *et al.*, 2010). Amikacin showed highest sensitivity in both ESBL and MBL producers.

S. aureus is a highly versatile and adaptable pathogen, causing a range of infections of varying severity affecting the skin, soft

tissue, respiratory system, bone, joints and endovascular tissues (Palalb ray *et al.*, 2011). MRSA burden is increasing worldwide in hospitals [healthcare-associated (HA)-MRSA] and in communities [community-associated (CA)-MRSA] (Ana L. Egea *et al.*, 2014).

Table.1 Various organisms isolated

Organisms	No. of organisms	Percentage (%)
<i>Staphylococcus aureus</i>	35	32.9
Coagulase negative <i>staphylococcus</i>	14	13.0
<i>P. aeruginosa</i>	17	15.8
<i>Escherichia coli</i>	12	11.2
<i>Klebsiella spp.</i>	14	13.0
<i>Enterobacter spp.</i>	12	11.2
<i>Proteus mirabilis</i>	3	2.9
Total	107	100

Table.2 Antibiotic susceptibility pattern of ESBL producers

Organisms	ESBL Producers No. (%)	Antibiotics							
		A No. (%)	G No. (%)	AK No. (%)	CF No. (%)	CO No. (%)	CE No. (%)	CA No. (%)	I No. (%)
<i>P. aeruginosa n-17</i>	12(70.5)	0(0)	3(25.0)	6(50.0)	5(41.6)	2(16.6)	0(0)	0(0)	9(75.0)
<i>E. coli n-12</i>	07(58.3)	0(0)	2(28.5)	4(57.1)	2(28.5)	2(28.5)	0(0)	0(0)	6(85.7)
<i>Klebsiella spp. n-14</i>	12(85.7)	0(0)	0(0)	9(75.0)	8(66.6)	0(0)	0(0)	0(0)	10(83.3)
<i>Enterobacter spp. n-12</i>	09(75.0)	0(0)	3(33.3)	2(22.2)	3(33.3)	3(33.3)	0(0)	0(0)	8(88.8)
<i>P. mirabilis n-3</i>	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total (%) n-58	40(68.9)	0(0)	8(20.0)	21(52.5)	18(45.0)	7(17.5)	0(0)	2(5.0)	33(82.5)

Table.3 Antibiotic susceptibility pattern of MBL producers

Organisms	MBL Producers No. (%)	Antibiotics							
		A No. (%)	G No. (%)	AK No. (%)	CF No. (%)	CO No. (%)	CE No. (%)	CA No. (%)	I No. (%)
<i>P. aeruginosa n-17</i>	4(23.5)	0(0)	0(0)	2(50)	0(0)	1(25.0)	0(0)	0(0)	1(25.0)
<i>E. coli n-12</i>	2(16.6)	0(0)	0(0)	0(0)	0(0)	1(50.0)	0(0)	0(0)	0(0)
<i>Klebsiella spp. n-14</i>	3(21.4)	0(0)	0(0)	1(33.3)	1(33.3)	0(0)	0(0)	0(0)	1(33.3)

<i>Enterobacter spp. n-12</i>	1(8.3)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)
<i>P. mirabilis n-3</i>	1(33.3)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
Total (%) n-58	11(18.9)	0(0)	0(0)	5(45.4)	1(9.0)	2(18.1)	0(0)	0(0)	3(27.2)

Table.4 Antibiotic susceptibility pattern of MRSA producers

Organisms	MRSA No. (%)	Antibiotics								
		A No. (%)	G No. (%)	AK No. (%)	CF No. (%)	CO No. (%)	E No. (%)	CD No. (%)	LZ No. (%)	VA No. (%)
<i>S. aureus n-35</i>	14(40)	0(0)	6(42.8)	11(78.5)	5(35.7)	7(50.0)	4(28.5)	5(35.7)	14(100)	14(100)

A – Ampicillin, G – Gentamicin, AK – Amikacin, CF – Ciprofloxacin, CO – Cotrimoxazole, CE – Cefotaxime, CA – Ceftazidime, I – Imipenem, E – Erythromycin, CD - Clindamycin, LZ – Linezolid, VA – Vancomycin

As the epidemiology of MRSA disease changes, including both community-and health care-associated disease, accurate information on the scope and magnitude of the burden of MRSA disease is needed to set priorities for prevention and control (Monina Klevens *et al.*, 2007).

Methicillin resistance in *S. aureus* is based on the production of an additional penicillin binding protein, PBP2 or PBP2a, which is mediated by the *mecA* gene (Stephen T Odonkor and Kennedy K Addo, 2011). MRSA strains are resistant to many different classes of antibiotics especially to beta-lactam group. In our study, MRSA was detected in 40% isolates of *S. aureus*. All MRSA isolates were sensitive to linezolid and vancomycin. With increasing isolation of MRSA producing isolates and wide spread use of vancomycin as a treatment option also increases the problem of vancomycin resistant *Staphylococcus aureus* (VRSA).

A simple screening test has been very useful

to screen this problem of drug resistance. The early detection of such drug resistant isolates may help in appropriate antimicrobial therapy and avoid the development and dissemination of these multidrug resistance strains.

In conclusion, there is high prevalence of beta lactamase and MRSA isolates in our study. Formulation of proper antibiotic policy and providing appropriate guidelines to prescribe antibiotics can prevent the spread of multidrug resistant organisms in the hospital as well as in the community.

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