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Pharmaceutical Drug Regulatory Affairs Journal
ISSN: 2642-6315

Isolation and Identification of Methicillin Resistant Staphylococci at Bethzatha Advanced Laboratory, Addis Ababa, Ethiopia

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Research Article

Volume 7 Issue 1

Received Date: February 08, 2024

Published Date: February 28, 2024

DOI: 10.23880/pdraj-16000154

Abstract

Methicillin resistant *Staphylococcus aureus* and coagulase negative staphylococci (CoNS) are widely spreading. So identification and antimicrobial susceptibility tests of methicillin resistant *S. aureus* and coagulase negative staphylococcus (CoNS) is critical to appropriately treat patients and control the increasing spread of these pathogens. Therefore the aim of the present work is to determine methicillin resistant S.aureus and CoNS from clinical samples sent to BethZatha Advanced Laboratory from different health institutions and Bethzatha Hospital, Addis Ababa, for bacterial isolation, identification and antimicrobial susceptibility. Bacterial isolation, identification and antimicrobial susceptibility testing were done using Microscan panel methods (Beckman Coulter, Brea, CA, USA) from May 2021 to February 2022 at Bethzatha advanced Medical Laboratory. The retrospective data was analysed to determine methicillin susceptibility of S.aureus and Coagulase negative staphylococci (CoNS). The finding shows 35% (40/114) *S. aureus* and 65.8% (75/114) coagulase negative staphylococci (CoNS) were isolated from different clinical specimens (urine, blood culture, pus and discharges). Out of 40 *S. aureus* isolates 30% (12/40) were methicillin resistant. Similarly, out of 75 CoNS, 52% (39/75) were methicillin resistant. Almost all methicillin resistant *S. aureus* and most CoNS isolates were multiply resistant to amoxicillin clavulnate, azithromycin, penicillin and ampicillin. Periodic surveillance and appropriate control measures and safety precautions of nosocomial and community acquired methicillin resistant multidrug resistant, *staphylococcus aureus*, and CoNS must be given prior attention.

Keywords: Methicillin Resistant; *Staphylococcus Aureus*; Coagulase Negative Staphylococci; Multi-Drug Resistance

Abbreviations: AMC: Amoxicillin; Azk: Azithromycin; CIP: Ciprofloxacin; Gm: Gentamicin; IP: Imipenem; LE: Levvofloxacin; TC: Tetracycline; TSM: Trimethoprim sulfamethoxazole; Am: Ampicillin; Dap: Daptomycin; CD: Clindamycin; ER: Erythromycin; OC: Oxacillin; TP: Tecioplanin; FM: Fosfomycin; VAN: Vancomycin; LZL: Linezolid; SYA: Synercid; P: Penicillin; FUA: Fusidic Acid.

Introduction

Staphylococci are gram positive bacteria most of which colonize human and animal skin as transitory or commensal flora [1]. Coagulase negative staphylococci (CoNS) have been frequently isolated as an agent of nosocomial infections in last twenty years although they are pathogens which have low virulence rate [2]. They are colonized in hospital environment and hospitalized patients' skin. Immunosuppressive therapies, invasive procedures, common usage of broad-spectrum antibiotics tend to result in bacterial infections. CoNS are an important agent that may lead to hospital acquired bacteraemia [3]. However when any bacteria are in the wrong site or other than their normal niche can cause infections. *Staphylococcus aureus* can be pathogenic if introduced into normally sterile sites such as

blood stream, urinary tract, cerebrospinal and other internal tissues.

Methicillin is a drug that was introduced to treat patients with infections that is caused by penicillin resistant *staphylococcus aureus* [4]. Methicillin, the first semisynthetic penicillinase resistant penicillin, was widely used initially until methicillin resistant *S aureus* (MRSA) was found in England in 1961 [3]. MRSA acquired drug resistance via incorporation of the *mecA* gene, which encodes an alternative penicillin-binding protein, PBP 2a or its ortholog into the chromosome. Shortly after the introduction of methicillin in clinical world to treat infections caused by penicillinase producing *S. aureus,* methicillin resistant *S.aureus* (MRSA) emerged and spread worldwide [4]. The high rate of methicillin resistance among *Staphylococcus aureus* has resulted into the increased interest for the use of other drugs such as clindamycin, vancomycin and oxacillin for treatment of infections caused by MRSA.

Methicillin resistance in *S. aureus* is mediated through an altered protein called low-affinity penicillin binding protein (PBP2a). PBP2a is encoded by mecA gene which is present in chromosomal mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec) specific site, encoding an alternative penicillin-binding protein that has low affinity for almost the entire class of β-lactam drugs including methicillin, oxacillin, and most cephem agents [5,6]. Thus, MRSA is defined as S. aureus isolates genetically containing mecA or mecC or phenotypically showing resistance to oxacillin conventionally or to cefoxitin [7]. Cefoxitin is a more potent inducer of mecA and disk diffusion tests using Cefoxitin reveal clearer endpoints that are easier to read than tests with oxacillin [8]. In the recent past, there have been multiple reports on the use of cefoxitin as a surrogate marker for detection of mecA. However, some clinical isolates are mecA-positive and oxacillin susceptible and defined as oxacillin-susceptible MRSA (OSMRSA). Due to possible association of MRSA with multiple antibiotic resistance and relatively difficult and higher cost of treatment, the accurate and rapid identification of MRSA is crucial in clinical world for timely management of the infections caused by this superbug. Thus the present work is to determine methicillin resistant *S. aureus* and coagulase negative staphylococci (CoNS) from different clinical specimens.

Materials and Methods

Specimen and Bacteriological Culture

Different clinical specimens (urine, blood culture, pus and discharges from different body sites) sent to Bethzatha Laboratory from different wards of Bethzatha Hospital and other Health Institutions in Addis Ababa for culture and antimicrobial susceptibility tests. These were cultured for isolation and identification of bacterial pathogens and testing antimicrobial susceptibility. The cultures were done on conventional culture media such as Blood agar, Nutrient Agar, Mannitol salt agar, Chocolate agar as recommended by Cheesbrough [9].

Bacterial Identification and Antimicrobial Susceptibility Test

Bacterial identification and antimicrobial susceptibility tests were done using Microscan panel identification methods (Beckman Coulter, Brea, CA, USA). Identification of Gram Positive organisms was done using dried positive COMBO panels. Microscan dried COMBO Panel is a Panel containing both dried biochemical reagents and antimicrobials. In Microscan COMBO Panels, susceptibility to different antimicrobials was tested by the minimum inhibitory concentration (MIC) methods, with break point referenced to CLSI (Clinical and Laboratory Standard Institute) guide line. According to MicroScan Panel identification methods, 3-4 pure bacterial colonies were picked from 18-24 hours' aerobic culture by means of a wand designed for holding bacterial material from primary isolation media mentioned above and inoculated into 30 ml of Prompt inoculation water (Beckman Coulter, Brea, CA, USA). Then the bacterial suspension was transferred into Seed Tray Inoculator D sets (Beckman Coulter, Brea, CA, USA). The COMBO panel wells' are inoculated from bacterial suspension in the Seed Tray using a device known as Microscan Renok (Beckman Coulter, Brea, CA, USA) which delivers 115 µL of broth suspension to each well. Arginine and urea containing wells were overlaid with the mineral oil as instructed by the company. Some reagents recommended by the manufacturer were added to the panels after incubating for 18 to 24 hours at 35°c aerobically before reading.

The panels were read by MicroScan AutoScan 4 automated reader (Beckman Coulter, Brea, CA, USA). The Microscan automated reader gives the identification for each bacterial biotype with probability scores. Results with high probability scores (>85%) were considered reliable while results with probability scores (<85%) "Unconfirmed". If the biochemical profile did not much any identification in Program's software database, the result generated was "very rare bio type".

The antimicrobial susceptibility test result included Cefoxitin screening and oxacillin susceptibility tests. Analysis of Cefoxitin screening and oxacillin resistant strains of staphylococci isolates was done from the retrospective data record.

Result

A total of 114 staphylococci, 40(35%) *S. aureus* and 75(65.8%) coagulase negative staphylococci (CoNS) were isolated from different clinical specimens The most dominant coagulase negative isolates were *S.haemolyticus*, 21% (16/75), *S.epidermidis*, 20%(15/75), and *S.auricularis,* 19% (14/75) (Table 1). *S. aureus* was most frequently 54% (26/48) isolated from pus followed by 34% (15/44) from blood cultures. The most frequent CoNS isolate from blood culture was *S. haemolyticus*, 36% (13/36) followed by *S.epidermidis*, 19% (7/36) (Table 1).

CoNS Isolates	Sample Types			
	Urine	Blood	Pus	Total
S. auricularis	5(27.8)	3(8.3)	6(28.6)	14(18.7)
S. intermedius	1(5.6)	3(8.3)	3(14.3)	7(13.7))
S. xylosus	-		1(4.8)	1(1.3)
S.hycus	-	-	3(14.3)	3(4)
S. haemolyticus	2(11)	13(36)	1(25)	16(21.3)
S.cohnni	1(5.6)	1(2.8)	1(4.8)	3(4)
S .epidermidis	4(40)	7(19.4)	4(19)	15(20)
S.canis	1(5.6)	-	-	1(1.3)
S.scium	4(22)	-	2(9.5)	6(8)
S.HOMINI	-	9(25)		9(12.0)
Total	18	36	21	75(100)

Table 1: Coagulase-Negative Staphylococci (Cons) Isolated from Different Clinical Samples.

S. auricularis was isolated most frequently, 28.6 % (6/21) followed by *S.epidermidis,* 19 % (4/21) from pus and body fluids. Out of 114 staphylococci isolates, 51(44.3%) were methicillin resistant (both cefoxitin screening positive and oxacillin resistant) (Table 2). Out of 40 *S. aureus* isolates, 30% (12/40) were methicillin resistant. Similarly, of 75 CoNS, 52% (39/75) were methicillin resistant. *S.hemolyticus*, *S.homini* and *S. epidermidis* 16/16(100%), 7/9 (78%) and 11/15(73%) were methicillin resistant respectively (Table 2).

Isolates	Samples				Cefoxitin Screening Positive and Oxacillin Resistant
	Urine	Blood	Pus and Body Fluid	Total	(Methicillin Resistant)
S. aureus	-	15(34)	25(54.3)	40(35)	12(30)
S. auricularis	5(29.4)	3(6.8)	6(12.5)	14(12.2)	2(14.3)
S. intermedius	1(5.9)	3(6.8)	36.25)	7(6.0)	1(14)
S. xylosus	-		1(2.3)	1(0.86)	1(100)
S.hycus	-		3(6.25)	3(2.6)	0(0)
S. haemolyticus	2(11.8)	13(29.5)	1(2.3)	16(13.9)	16(100)
S.cohnni	1(5.9)	1(2.3)	1(2.3)	3(2.6)	0(0)
S. epidermidis	4(23.5)	7(15.9)	4(8.3)	15(13.0)	11(73)
S.scium	4(23.5)	-	2(4.2)	6(5.2)	1(17)
S.HOMINI	-	9(20.4)		9(7.8)	7(78)
Total	17	51	46	114	51(44.7)

Table 2: Methicillin Resistant *S. Aureus* and Coagulase –ve Staphylococci (Cons) Isolated from Clinical Samples.

Table 3 shows antimicrobial susceptibility patterns of methicillin resistant staphylococci isolated from different clinical samples. All methicillin resistant *S. aureus* isolates 12/12(100%) were resistant to amoxicillin clavulnate, azithromycin, ampicillin and 11/12(91.7%) to oxacillin and clindamycin. Among CoNS, *S. haemolyticus*, 16/16(100%)

was resistant to amoxicillin clavulnate, oxacillin and 15/16(93.7%) to ciprofloxacin, ampicillin and imipenem. Simillarly all 11/11(100%) methicillin resistant *S. epidermidis* were resistant to amoxicillin clavulnate, ampicillin, oxacillin and penicillin (Table 3). On the other hand 9/12(75%) of *S. aureus* isolates were susceptible to Daptomycin, vancomycin, Fusidic, linozolin and all, 16/16 (100%) *S. haemolyticus* were susceptible to teicoplanin and vancomycin.

Anti micrbial Agens	S. aureus		S intermedius		S. xylosus		S. homini		S. haemolyticus		S. scuri		S. Epidermidis	
	N=12		N=1		N=1		N=7		N=16		N=1		N=11	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
AMC/CL	0(0)	12 -100		1(100)		1 -100	0(0)	7(100)	0	16 -100		1(100)	0(0)	11 -100
AZK	0(0)	12 -100		1(100)		1 -100	3(42.9)	4(57.1)	2(12.5)	14 -87.5		1(100)	11(9)	10(91)
CIP		10 -83		1(100)		1(100)	2(28.6)	2(28.6)	1(6.3)	15 -93.8		1(100)	4(36.4)	6(54.5)
GM	6(50)	5 -41.7		1(100)		0	6(85.7)		3(18.8)	13 -81.3		1(100)	3(27.3)	7(63.6)
IP	2(16.7)	9(75)		1(100)		1(100)	5(71.4)		1(6.3)	15 -93.8		1(100)	0(0)	10(91)
LE	3(25)	9(75)		1(100)		1(100)	5	2(28.6)	3(18.8)	13 -81.3		1(100)	4(36.4)	7(63.6)
TC	4(33.3)	8 -66.7		1(100)			2(28.6)	0(0)	4(25)	12(75)		1(100)	1(9)	10(91)
TSM	7(58)	5 -41.7		1(100)			4(57.1)	0(0)	10 -62.5	6(37.5)		1(100)	3(27.3)	8(72.7)
AM		12 -100	-	1(100)		1 -100	-	6(85.7)	1(6.3)	15 -93.8			0(0)	11 -100
DAP	9(75)	0		1(100)		1	5(71.4)		12(75)				7(63.6)	0
CD	1	11 -91.7		1(100)			6(85.7)		8(50)	8(50)		1(100)	4(36.4)	7(63.6)
ER	2(16.7)	9(75)	-	1(100)		1 -100		4(57.1)	4((25)	12(75)		1(100)	3(27.3)	7(63.6)
OC	1(8.3)	11 -91.7	-	1(100)		1(100)	0(0)	7(100)	0(0)	16 -100		1(100)	0(0)	11 -100
TP	8(66.7)	4 -33.3	1 -100	0(0)			6(85.7)	0(0)	16 -100	0		1(100)	7(63.6)	4(36.4)
mox	3(25)	7(58)		1(100)			3(42.9)	2(28.6)	4(25)	12(75)		1(100)	5(45.5)	5(45.5)
FM		8 (66.7	1(100)	0(0)		1(100)	6(85.7)	0(0)				1(100)	7(63.6)	4(36.4)
FUA	9(75)	3(25)	1(100)			1(100)		1(14.1)						
VAN	9(75)	-		1(100)		1(100)	6(85.7)	1(14.1)	16 -100			1(100)	6(54.5)	4(36.4)
LZL	9(75)	3(25)	1(100)	0(0)		1(100)	4(57.1)	0	0(0)	2(12.5)		1(100)	7(63.6)	3(27.3)
SYA	8(66.7)	4(33.3)	1(100)			1(100)	6(85.7)	1(14.1)	15(75)	1(6.3)		1(100)	6(54.5)	3(27.3)
P	1(8.3)	11(91.7)	-	1(100)		1(100)		7(100)				1(100)	0(0)	11 -100

Table 3: Antimicrobial Susceptibility Patterns of Methicillin Resistance *S. Aureus* and Coagulase Negative Staphylococci Isolates from Clinical Samples.

Discussion

In the present study methicillin resistant *S.aureus* was frequently isolated from pus and discharges and blood culture. The rate of methicillin resistant S. aureus, 30% in the present study is comparable to 28.3% rate of MRSA isolation from Desie hospital, North West Ethiopia [10], but lower compared to the national pooled rate 32.5% [6], 68% reported from Awasa, southern Ethiopia [11] and 35.6% from Tikur Anbesa Hospital Addis Ababa, Ethiopia [12]. However 30% methicillin resistant rate of *S.aureus* in the present study is higher than 23% methicillin resistance reported from Jimma, South west Ethiopia [13], 13.2% from Debrmarkos referral hospital, north west Ethiopia [14]. A study from Arbaminch Southern Ethiopia Mekuriya E, et al. [15] reported only 7.4% methicillin resistant *S. aureus* from nasal colonization which is relatively lower rate than most of the above studies. The variation observed could be due to the source of bacteria, the nature of the study participants, the laboratory methods used, and the study methods applied. Most (92%- 100%) of methicillin resistant *S.aureus* in the present study were multiply resistant to commonly used antibiotics, amoxicillin clavulnate, azithromycin, ampicillin, penicillin, clindamycin, ciprofloxacin and erythromycin. Multi-drug resistance to penicillin, ampicillin, amoxicillin, and ciprofloxacillin of MRSA isolates has been reported by many other workers from Ethiopia and elsewhere [16]. On the other hand, most (75%, 9/12) methicillin resistant *S.aureus* in the present study were susceptible to daptomycin, vancomycin, linezolid and fusidic acid. Dhunggel S, et al. [17] studied the prevalence and antimicrobial susceptibility of methicillin resistant *staphylococcus aureus* from tertiary heart care centre, Nepal, and found that none of the isolates was resistant to vancomycin. A similar pattern of susceptibility was documented by Nystinga J, et al. [16] from Kenya. In the present study Coagulase negative staphylococci (CoNS) were predominantly isolated from blood culture and pus and discharges and were frequently methicillin resistant. Other workers have also reported that Coagulase-negative staphylococci are among the most frequently isolated microorganisms in blood cultures [18]. The commonest CoNS isolated from blood cultures were *S.haemolyticus, S.homini* and *S.epidermidis. I*n the present study, *these* CoNS were most resistant to azithromycin, ampicillin, and penicillin. Researchers from elsewhere also reported that infection-associated with CoNS from healthcare environments are typically characterized by pronounced antimicrobial resistance (AMR) including both methicillin and multidrug-resistant isolates [17]. Asante J, et al. [18] has documented a similar observation from South Africa. Coagulase negative staphylococci in the present study were highly susceptible to daptomycin, teicoplanin and vancomycin. The high susceptibility of CoNS to vancomycin in present study agrees with reports from Osaka city Yamada K, et al. [19] and south India [20]. Coagulase negative staphylococci are mostly opportunistic and are increasingly associated with nosocomial infections, especially among the immunocompromised and those with invasive medical devices, and raise a significant health problem [21].

Conclusion and Recommendation

Multidrug resistant bacterial infection has become global problem making treatment option restricted. The situation is more challenging to low and middle economy countries. In the present study isolation of methicillin resistant *Staphylococcus aureus* and coagulase negative staphylococci is considerably high, besides, these strains showed extreme resistance to commonly prescribed antimicrobials such as penicillin, ampicillin, erythromycin and azithromycin. In principle, appropriate use of antibiotics, applying safety precautions are the key to reduce the spread of multidrug resistant bacteria including methicillin resistant staphylococci and other bacteria. So Periodic surveillance and appropriate control measures and safety precautions of nosocomial and community acquired MRSA and CoNS must be given prior attention. Furthermore, strengthening microbiology laboratory with necessary reagents, equipment and skilled man power is critical if we are to control the spread of these pathogens.

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