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PREVALENCE OF GRAM-POSITIVE BACTERIA IN JEDDAH, KINGDOM OF SAUDI ARABIA: STUDY OF ANTIMICROBIAL RESISTANCE PATTERNS AND MOLECULAR TYPING

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ABSTRACT

Constant surveillance of microbial pathogens is essential for combating infectious diseases which account for at least a quarter of all illnesses. This prospective study was designed to provide information on the incidence of Gram-positive bacterial infections in King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia. A total of 98 Gram-positive isolates were obtained and identified. Their antibiotic susceptibilities were documented against several antibiotics. All methicillin-resistant *Staphylococcus aureus* (MRSA) were examined for the existence of the *Staphylococcus aureus* specific gene and *mecA* gene by polymerase chain reaction. The most common individual isolates were staphylococci (62.2%) followed by enterococci (30%), then streptococci (0.05%). Twenty nine percent of the isolates were recovered from wounds and the highest incidence was in intensive care units (30.61%). Twenty percent of MRSA isolates were multidrug resistance. Glycopeptide resistances were detected in two isolates of *Enterococcus faecium.* All strains were sensitive to linezolid and tigycycline. Molecular technique found that all the isolates of MRSA had *S. aureus* specific gene and *mecA* gene. In conclusion, the incidence of resistance in Gram-positive cocci causing infections in KAUH is an increasing problem and molecular techniques using *mecA* gene can be used to detect MRSA.

KEYWORDS: Antimicrobial resistance, MRSA, molecular typing, *S.aureus*, *mecA* gene, Gram positive.

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INTRODUCTION

Globally improvements in human health are indicated with increase in longevity and reduction in infant mortality. However, infectious diseases continue to be a major challenge. Despite great strides in diagnosis and treatment the emergence of new pathogens in addition to drug resistant organisms have posed new threats. Gram-positive cocci are a heterogenous group of bacteria which include many important human pathogens such as *Staphylococcus*, *Streptococcus* and *Enterococcus* and they are producing a wide spectrum of life threatening diseases including infections of skin, soft tissues, bones, urinary tract, respiratory tract and opportunist infections. Some species are associated with specific niches. Among these, *Staphylococcus aureus* (*S. aureus*), especially multidrug resistance isolates (MRSA) are the most virulent pathogens. Moreover, hospital environments particularly encourage the evolution of new and many drug resistant strains[1].The emergence of MRSA has presented clinicians with a major challenge. Importance of epidemiological surveys and monitoring antimicrobial susceptibility was not emphasized enough. It is well known that, Methicillin resistance in *S. aureus* is controlled by *mecA* gene which codes for a novel penicillin binding protein (PBP) and are originally associated with nosocomial infections. These strains are now known becoming increasingly important in community acquired infections [2,3,4]. *Streptococcus agalactiae* (*St. agalactiae*), initially recognized as a cause of puerperal sepsis, is now known to produce life threatening diseases such as meningitis, pneumonia and septicemia in neonates in addition to a range of diseases in other patients. Most isolates of *St. agalactiae* are sensitive to penicillin but resistance to tetracycline and erythromycin has been reported[5]. Enterococococci originating from the normal gastrointestinal tract flora traditionally were considered to have low virulence. However, vancomycin resistant strains which were first reported in patients with renal failure are now frequently reported among nosocomial infections and in patients with other pre-disposing factors[6]. Antibiotic resistance among enterococci results from mutation and acquisition of genetic material from other species. High levels have been reported in United State (76%) and Europe (40%)[7]. Prevalence of drug resistant strains is known to differ globally and is known to be affected by numerous factors including, misuse of antibiotics, non-compliance by patients, antibiotic prescribing policies and infection control procedures being used[8, 9]. It is therefore imperative that there is continual surveillance of such strains in all large health care settings and within different countries worldwide. The aim of this study was to provide information on the trends observed amongst clinical infections with Gram-positive bacteria with respect to the prevalence, sites commonly implicated and the antimicrobial susceptibility patterns.

MATERIALS AND METHODS

(i) Setting and Design

This prospective study was carried out at King Abdulaziz University Hospital in Jeddah, Saudi Arabia which is the only university hospital in Jeddah. It is a tertiary care, teaching hospital with 800 beds. It provides care to adult and pediatric patients in medical, surgical, intensive care, obstetrics, gynecology and renal units. This cross sectional study was carried out from July 15th, 2011 to August 15th, 2011.

(ii) Data collection

For each isolate used in this study, information collection including patient demographics (name, age, sex and nationality), hospital wards where patients stayed, date of admission and discharge, date of specimen collection, the source of specimen, mode of acquisition (nosocomial or community acquired), co-morbidities, surgery and other invasive procedures, presence of foreign devices, history of hospitalization or antibiotic therapy during the last 6 months and outcome were obtained.

Isolates were considered to be hospital acquired if they were collected more than 72 hours from admission and nosocomial if they were recovered after that period[10].

(iii) Bacterial isolates

Ninety-eight Gram-positive isolates from different collected specimens (urine, blood, wound and respiratory secretions) were cultured on sheep blood and mannitol salt agar plates (Saudi Prepared Media Laboratory, SPML, Riyadh) using sterile disposable loops. Plates were incubated at 35-37ºC for 24-48 h. Preliminarily identification of isolates was performed by conventional methods[11] and manufacturer´s instructions including: colonial morphology, culture characteristics on agar media, Gram-staining and biochemical reaction such as: catalase test (Oxoid, UK), coagulase test (Oxoid, UK), DNase test (Oxoid, UK), nitrocefin test, bile esculin agar test (SPML, Riyadh) and serological reaction such as: agglutination test for their antigen (bioMérieux, France). Further biochemical identification of Gram-positive cocci to species level was confirmed by performing ID-GP (Gram-positive colorimetric identification card, bioMérieux, France) on the Vitek2® Automated Microbiology System, according to the manufacturer's instructions.

(iv) Antimicrobial susceptibility testing

The susceptibility of isolates to antimicrobial agents was screened by disc diffusion method (Kirby-Bauer) on Mueller-Hinton (MH) agar (SPML, Riyadh), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2011)[12]. All discs were supplied by Oxoid (UK). *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* (*E. faecalis*) ATCC 29212 were used as control for Gram-positive bacteria.

(v) Detection of methicillin resistance in S. aureus by phenotypic methods

Susceptibility to cefoxitin (30µg) and oxacillin (1µg) was performed using disk diffusion method to determine resistance against methicillin for *S. aureus*. The zones of inhibition for oxacillin and cefoxitin were measured according to the CLSI 2011 guidelines[12].

(vi) Determination of minimum inhibitory concentrations (MICs)

Minimum inhibitory concentrations were determined using Vitek 2® Automated Microbiology System. Vitek 2 Gram-positive MIC cards (AST-P580/AST-P586), used according to the manufactures instructions. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as control.

(vii) Detection of oxacillin resistance by genotypic method (DNA extraction and isolation)

400 µl of buffer ATL was added to suspension of MRSA previously identified by antimicrobial susceptibility tests. After heating, 400 µl buffer AL and 50 µL of proteinase K was added. Four hundred microliter ethanol was mixed carefully. After the mixture was applied to mini spin column, 700 µl buffer AW1, 700 µl buffer AW2 and 140 µl buffer AE were added in sequence and centrifuged. The product was stored in the freezer until used.

(viii) Amplification of S. aureus specific sequence gene and mecA gene

After DNA extraction, two primers [The 3-end region of the *S. aureus* specific gene was amplified using a forward primer 5'- AAT CTT TGT CGG TAC ACG ATATTC TTC ACG -3' and a reverse primer, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3'. While the 3-end region of the *mecA* gene was amplified using a forward primer 5'- AAA ATC GAT GGT AAA GGT TGG C - 3' and a reverse primer, 5'- AGT TCT GCA GTA CCG GAT TTG C-3' (TIB, Molbiol, Berlin, Germany)] were used to amplify both *S. aureus* specific sequence gene and *mecA* gene using polymerase chain reaction (PCR). Polymerase chain reaction was performed in a 50 µl volume. Ten µl of DNA template was added to 40 µl of PCR mixture (25 µl of Master mix Maxima® HOT Start Taq DNA polymerase supplied in 2.5 units HotStar Taq DNA polymerase, 1X hot start PCR buffer, 200

µM of each dNTP and two dyes for direct loading on agarose, 13 µl of nuclease-free water and 1 µl of each primer). DNA amplification was a carried out in a thermal cyclers (Perkin-Elmer, USA) with the following thermal cycling profile: an initial denaturation step at 95ºC for 15 min. followed by 40 cycles of amplification (denaturation at 94ºC for 45 sec., annealing at 55ºC for 45 sec., and extension at 72ºC for 1 min), ending with a final extension step at 72ºC for 10 min. after the last cycle, the products were stored at 4ºC.

(ix) Pulsed-Field Gel Electrophoresis

Polymerase chain reaction products were tested for positive amplification by agarose gel electrophoresis. The DNA bands were visualized by ultraviolet light and photographed. The individual lanes compared with known molecular weight standard (GelPilot® 100bp ladder plus ladder 100 lanes, QIAGEN) and two previous positive controls (one *S. aureus* with specific gene and other MRSA with specific gene and *mecA* gene). The sizes of amplified fragments were determined by calculating their relative mobilities to the molecular weight standard and analysis of PCR products.

RESULTS

During the study period, 98 clinical isolates were collected from 91 patients. Forty four over 91 patients (48.4%) were male and 47/ 91 (51.6%) were female. The ages of the patients ranged from less than one year to 100 years, with a mean age of 38.04± 26.68 years. Thirty-eight (41.76%) patients were Saudi and 53(58.2%) were non-Saudi. Forty nine percent of isolates were community acquired and 51.02% were nosocomial. Gram-positive cocci isolated from inpatients accounted for 83.67% and 16.33% originated from outpatients. Seventy six out of 91 patients (83.5%) had underlying diseases or at least one co-morbidity disease such as diabetes, renal failure, cardiac disease, pulmonary disease, immune disorders and other infections. Other factors influencing the prevalence were recent hospitalization (before 6 month), recent surgery and previous antibiotic treatment with agents such as cephalosporins, metronidazole and vancomycin. (Gram-positive cocci collected during the study period, the variety of species and their percentage is presented in table 1.

Table 1
Number and percentage of Gram-positive bacteria isolated from KAUH

Bacterial isolates	No. of isolates	%
Methicillin-sensitive *Staphylococcus aureus* (MSSA)	19	19.39
Methicillin-resistant *Staphylococcus aureus* (MRSA)	10	10.20
Staphylococcus epidermidis	3	3.06
Staphylococcus haemolyticus	14	14.29
Staphylococcus capitis	7	7.14
Staphylococcus hominis ssp *hominis*	4	4.08
Staphylococcus warneri	2	2.04
Staphylococcus gallinarum	1	1.02
Staphylococcus lugdunensis	1	1.02
Micrococcus luteus	1	1.02
Aerococcus viridians	1	1.02
Kocuria kristinae	1	1.02
Enterococcus faecalis	21	21.43
Enterococcus faecium	5	5.10
Enterococcus gallinarum	1	1.02
Enterococcus raffinosus	1	1.02
Enterococcus hirae	1	1.02
Streptococcus agalactiae	4	4.08
Streptococcus sanguinis	1	1.02
Total	**98**	**100**

Table 1 showed that staphylococci were isolated with the highest frequently (62.2%) followed by enterococci (29.6%) and streptococci (0.05%), other genera namely; *Micrococcus luteus*, *Aerococcus viridans* and *Kocuria kristinae* were detected infrequently. Among the 61 staphylococci (31.15%) were methicillin sensitive *S. aureus* (MSSA), (16.39%) were MRSA and (52.46%) were coagulase-negative staphylococci (CoNS). Whilst among the enterococci isolates (72.41%) were *E. faecalis* and (17.24%) accounted for *E. faecium*. Table 2 shows the distribution of isolates among the different hospital units.

Table 2
Prevalence of Gram-positive isolates among the different hospital units of KAUH

		Wards						
Bacterial isolates	**No. of isolates**	**Intensive care units (ICU, PICU,NICU)**	**Medical**	**Pediatric**	**Surgical**	**Emergency room**	**Outpatient**	**Others***
MSSA	19	8	1	1	1	5	2	1
MRSA	10	5	1	0	0	2	1	1
S. epidermidis	3	1	0	1	0	0	0	1
S. haemolyticus	14	1	2	1	0	4	3	3
S. capitis	7	4	0	0	1	0	1	1
S. hominis ssp *hominis*	4	3	0	0	0	0	0	1
S. warneri	2	1	0	1	0	0	0	0
S. gallinarum	1	0	0	0	0	0	1	0
S. lugdunensis	1	0	0	0	0	0	1	0
M. luteus	1	0	0	0	0	0	1	0
A. viridans	1	0	0	0	0	1	0	0
K. kristinae	1	1	0	0	0	0	0	0
E. faecalis	21	3	3	1	5	3	2	4
E. faecium	5	3	2	0	0	0	0	0
E. gallinarum	1	0	0	0	0	0	0	1
E. raffinosus	1	0	0	0	0	0	1	0
E. hirae	1	0	0	0	0	1	0	0
St. agalactiae	4	0	1	0	1	0	2	0
St. sanguinis	1	0	0	0	0	0	1	0
Total	**98**	**30**	**10**	**5**	**8**	**12**	**16**	**13**

Others: private ward and operating room

Prevalence of MRSA (50%), MSSA (42.1%), and CoNS (31.5%) were the highest amongst ICUs patients. Incidence of *E. faecalis* was greater in the surgical unit (23.81%) while isolation of *E. faecium* was the most frequent in the ICUs (60%).Table 3 showed that Gram-positive bacteria were most frequently isolated with respect to different specimen types. The prevalence of Gram-positive bacteria was the highest in wound and abscess specimens (28.57%), followed by blood (24. 49%) and urine (22.45%) samples. Both MSSA and MRSA were preponderant in wound and abscess specimens with incidence percentage of 36.84% and 50%, respectively, while CoNS were mostly recovered from blood with incidence percentage of 50%. Furthermore, *Enterococcus* spp. and *St. agalactiae* were more frequently isolated from urine specimen (44.8%) and (50%) respectively.

Table 3
Distribution of Gram-positive isolates in various clinical specimens in KAUH

Bacterial isolates	No. of isolates	Specimens				
		Blood	Wound and abscess	Urine	Respiratory secretion	Others*
MSSA	19	3	7	3	5	1
MRSA	10	0	5	1	4	0
S. epidermidis	3	1	0	0	1	1
S. haemolyticus	14	5	3	2	0	4
S. capitis	7	5	2	0	0	0
S. hominis ssp *hominis*	4	4	0	0	0	0
S. warneri	2	1	1	0	0	0
S. gallinarum	1	0	0	1	0	0
S. lugdunensis	1	0	1	0	0	0
M. luteus	1	1	0	0	0	0
A. viridans	1	0	1	0	0	0
K. kristinae	1	1	0	0	0	0
E. faecalis	21	2	4	11	1	3
E. faecium	5	1	2	0	1	1
E. gallinarum	1	0	1	0	0	0
E. raffinosus	1	0	0	1	0	0
E. hirae	1	0	0	1	0	0
St. agalactiae	4	0	1	2	0	1
St. sanguinis	1	0	0	1	0	0
Total	**98**	**24**	**28**	**23**	**12**	**11**

Others: Eye swab, genitourinary swab, body fluids, catheter tip

Fifty three out of 61 staphylococcal strains (86.9%) were β-lactamase positive.

The antimicrobial resistance of tested isolates against different antibiotics were done by disc diffusion and MIC methods as shown in table 4 and 5

Table 4
Antimicrobial resistance patterns of Gram-positive cocci by disc diffusion method

Antibiotics / Bacterial isolates	Number of resistant isolates/Number of isolates (n/N) (%)						
	MSSA	**MRSA**	**CoNS**	***E. faecalis***	***E. faecium***	**Other *Enterococcus* spp.**	***Streptococcus* spp.**
Ampicillin (AMP)	16/19	10/10	27/32	0	5/5	1/3	0
	84.21	100	84.37	0	100	33.33	0
Penicillin (P)	16/19	10/10	28/32	2/21	5/5	1/3	0
	84.21	100	87.5%	9.52	100	33.33	0
Ampicillin/sulbactam (SAM)	NA	NA	NA	0	5/5	0	0
				0	100	0	0
Amoxicillin-clavulanic acid (AMC)	1/19	8/10	10/32	0	5/5	0	0
	5.26	80	31.25	0	100	0	0
Piperacillin (PRL)	3/19	9/10	24/32	0	5/5	1/3	0
	15.79	90	75%	0	100	33.33	0

Piperacillin-tazobactam (TZP)	0 0	6/10 60	5/32 15.62	0 0	5/5 100	0 0	0 0
Imipenem (IPM)	NA	NA	NA	0 0	5/5 100	0 0	0 0
Oxacillin (OX)	0 0	10/10 100	25/32 78.12	NA	NA	NA	NA
Cefoxitin (FOX)	0 0	10/10 100	25/32 78.12	21/21 100	5/5 100	3/3 100	0 0
Cefuroxime (CXM)	0 0	8/10 80	17/32 53.12	21/21 100	5/5 100	3/3 100	0 0
Vancomycin (VA)	0 0	0 0	0 0	0 0	2/5 40	0 0	0 0
Teicoplanin (TEC)	0 0	0 0	0 0	0 0	2/5 40	0 0	0 0
Azithromycin (AZM)	3/19 15.79	3/10 30	21/32 65.62	NA	NA	NA	NA
Clarithromycin (CLR)	3/19 15.79	3/10 30	21/32 65.62	NA	NA	NA	NA
Erythromycin (E)	3/19 15.79	3/10 30	20/32 62.5	9/21 42.86	5/5 100	1/3 33.33	0 0
Clindamycin (DA)	1/19 5.26	3/10 30	7/32 21.87	21/21 100	5/5 100	3/3 100	0 0
Chloramphenicol (C)	0 0	0 0	0 0	6/21 28.57	0 0	0 0	0 0
Fusidic acid (FD)	2/19 10.53	2/10 20	18/32 56.25	2/21 9.5	0 0	0 0	0 0
Gentamicin (CN)	1/19 5.26	4/10 40	19/32 59.37	NA	NA	NA	NA
Tobramycin (TOB)	3/19 15.79	3/10 30	18/32 56.25	NA	NA	NA	NA
Linezolid (LZD)	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Minocycline (MH)	0 0	0 0	0 0	15/21 71.43	1/5 20	1/3 33.33	0 0
Tetracycline (TE)	3/19 15.79	3/10 30	7/32 21.87	10/21 47.62	3/5 60	1/3 33.33	4/5 80
Tigycycline (TGC)	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Mupirocin (MUP)	0 0	2/10 20	2/32 6.25	NA	NA	NA	NA
Norfloxacin (NOR)	2/19 10.53	3/10 30	12/32 37.5	6/21 28.57	5/5 100	0 0	0 0
Ciprofloxacin (CIP)	2/19 10.53	3/10 30	12/32 37.5	6/21 28.57	5/5 100	0 0	0 0
Levofloxacin (LEV)	2/19 10.53	3/10 30	8/32 25	6/21 28.57	5/5 100	0 0	0 0
Moxifloxacin (MFX)	1/19 5.26	3/10 30	6/32 18.75	6/21 28.57	5/5 100	0 0	0 0
Nitrofurantoin (F)	0 0	0 0	2/32 6.25	0 0	0 0	0 0	0 0
Rifampicin (RD)	1/19 5.26	0 0	3/32 9.37	15/21 71.43	5/5 100	1/3 33.33	0 0
Trimethoprime/sulfamethoxazole (SXT)	0 0	2/10 20	8/32 25%	6/21 28.57	5/5 100	0 0	0 0

NA: Not applicable

Table 5
Antimicrobial resistance patterns of Gram-positive cocci by MIC method

	Number of resistant isolates/Number of isolates (n/N) %						
Bacterial isolates Antibiotics	**MSSA**	**MRSA**	**CoNS***	***E. faecalis***	***E. faecium***	**Other *Enterococcus* spp.**	***Streptococcus* spp.**
Ampicillin (AMP)	NA	NA	NA	0 0	5/5 100	0 0	0 0
Penicillin (P)	16/19 84.21	10/10 100	29/31 93.55	2/21 9.52	5/5 100	0 0	0 0
Ampicillin/sulbactam (SAM)	NA	NA	NA	0 0	5/5 100	0 0	0 0
Imipenem (IPM)	NA	NA	NA	0 0	5/5 100	0 0	0 0
Oxacillin (OX)	0 0	10/10 100	25/31 80.64	NA	NA	NA	NA
Cefoxitin (FOX)	0 0	10/10 100	25/31 80.64	NA	NA	NA	NA
Cefuroxime (CXM)	NA	NA	NA	21/21 100	5/5 100	2/2 100	0 0
Vancomycin (VA)	0 0	0 0	0 0	0 0	2/5 40	1/2 50	0 0
Teicoplanin (TEC)	0 0	0 0	0 0	0 0	2/5 40	0 0	0 0
Fosfomycin (FOS)	0 0	0 0	24/31 77.42	NA	NA	NA	NA
Erythromycin (E)	2/19 10.53	3/10 30	18/31 58.06	9/21 42.86	5/5 100	0 0	0 0
Clindamycin (DA)	1/19 5.26	3/10 30	8/31 25.81	21/21 100	5/5 100	2/2 100	4/4 100
Inducible Clindamycin Resistance	0 0	2/10 20	1/31 3.2	NA	NA	NA	NA
Fusidic acid (FD)	2/19 10.53	2/10 20	15/31 48.39	NA	NA	NA	NA
Gentamicin (CN)	1/19 5.26	4/10 40	19/31 61.29	NA	NA	NA	NA
Tobramycin (TOB)	3/19 15.79	4/10 40	21/31 67.74	NA	NA	NA	NA
Gentamicin (high-level) (CNH)	NA	NA	NA	12/21 57.14	2/5 40	0 0	NA
Streptomycin (high-level) (SH)	NA	NA	NA	6/21 28.57	4/5 80	0 0	NA
Linezolid (LZD)	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Tetracycline (TE)	3/19 15.79	3/10 30	13/31 41.93	15/21 71.43	3/5 60	1/2 50	3/4 75
Tigycycline (TGC)	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Mupirocin (MUP)	0 0	2/10 20	1/31 3.22	NA	NA	NA	NA
Quinupristin/dalfopristin (Q/D)	NA	NA	NA	21/21 100	0 0	1/2 50	0 0
Levofloxacin (LEV)	2/19 10.53	3/10 30	6/31 19.35	6/21 28.57	5/5 100	0 0	0 0
Moxifloxacin (MFX)	2 10.53	3/10 30	6/31 19.35	6/21 28.57	5/5 100	0 0	0 0
Nitrofurantoin (F)	0 0	0 0	0 0	0 0	0 0	0 0	0 0
Rifampicin (RD)	0 0	1/10 10	4/31 12.90	NA	NA	NA	NA
Trimethoprime/sulfamethoxazole (SXT)	1/19 5.26	3/10 30	7/31 22.58	6/21 28.57	5/5 100	1/2 50	0 0

NA: Not applicable

****NB: The MIC of Staphylococcus gallinarum not applicable with Vitek 2 Gram-positive (AST -P580) card.***

Resistance to oxacillin and cefoxitin was detected in 34.48% of *S. aureus* isolates. Resistance to penicillin and ampicillin was detected in 84.2% of MSSA isolates. Resistance to other antibiotics was ranged from 5.26 to 15.79%. Methicillin-resistant *S. aureus* were highly resistant to penicillin (100%), ampicillin (100%), amoxicillin-clavulanic acid (80%), piperacillin (90%), and piperacillin-tazobactam (60%). Twenty percent of MRSA isolates were multidrug resistant (MDR) (namely; resistant to penicillin and oxacillin plus 3 or more of the following agents: erythromycin, clindamycin, gentamicin and tetracycline). Mupirocin resistance was detected in two MRSA strains (20%) and one CoNS (with MIC ≥ 8 µg/ml). All MSSA and MRSA were sensitive to vancomycin, teicoplanin, linezolid, tigycycline, nitrofurantoin and chloramphenicol. Resistance to oxacillin was common at 81.25% in coagulase-negative staphylococci. Moreover, 69.23% of these isolates were also resistant to gentamicin. However, all oxacillin-resistant CoNS strains were susceptible to vancomycin. On the other hand, inducible clindamycin resistance (ICR) was demonstrated in three (4.9%) of staphylococcal isolates, the incidence of ICR was 2 (20%) for MRSA and 1 (3.2%) for CoNS.Three strains (10.3%) of *Enterococcus* spp. were resistant to vancomycin. Vancomycin and teicoplanin resistance were detected in two isolates (40%) consisting of *E. faecium* (MIC >32 µg/ml, Van A phenotype) derived from medical unit, and an isolate of *E. gallinarum* which was intermediate resistance to vancomycin (MIC, 4 µg/ml) and sensitive to teicoplanin (MIC ≤ 0.5 µg/ml, Van C phenotype). The VRE isolates were also resistant to several antibiotics including erythromycin, ciprofloxacin and high-level of aminoglycosides. *Enterococcus faecalis* and *E. faecium* were both resistant to high levels of gentamicin (57.14%) and (40%) and to streptomycin (28.57%) and (80%), respectively. All strains of enterococci were susceptible to linezolid, nitrofurantoin and tigycycline. Moreover, all strains of *St. agalactiae* were susceptible to most used antibiotics such as; penicillin, ampicillin and vancomycin, while, they were resistant to clindamycin and tetracycline. Amplification of *S. aureus* specific sequence gene and *mecA* gene:Polymerase chain reaction product of *S. aureus* specific gene and *mecA* gene were applied and visualized using UV light. Clear bands were found confirming that all MRSA isolates contained *mecA* gene. A molecular weight standard was applied to determine the molecular weight of the tested genes (Figure 1, 2). No visualized band was found for PCR product of the negative control (MSSA) using the primer of *mec*A gene. All MRSA isolates have *S. aureus* specific gene and *mecA* gene in their PCR products which confirm that all the isolates were MRSA.

Agarose gel electrophoresis

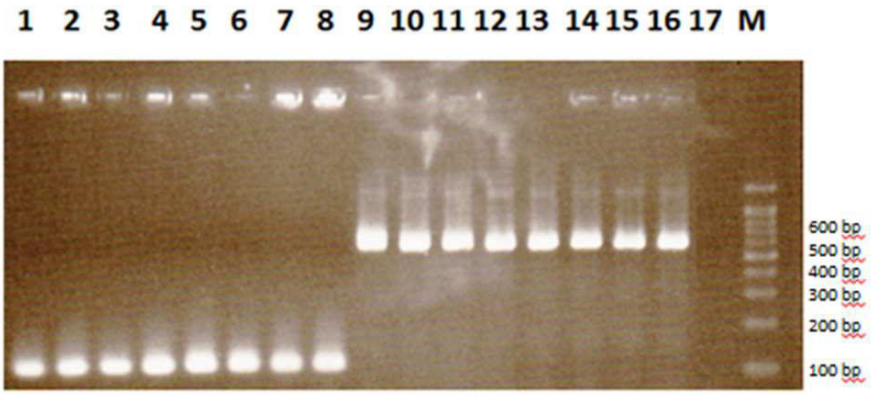

Figure 1

Detection of *S. aureus* specific gene and *mecA* gene by PCR. Lane 1-7; PCR product of seven *S. aureus* isolates amplified using *S. aureus* specific gene primer (amplicon size 107 bp); Lane 8 positive *S. aureus* control with specific gene; Lane 9-15, PCR product of seven *S. aureus* isolates amplified using *mecA* gene primer (amplicon size 532 bp); Lane 16, positive MRSA control with specific and *mecA* gene; Lane 17, negative control (MSSA) and M, 100-bp molecular weight standard (marker).

Agarose gel electrophoresis

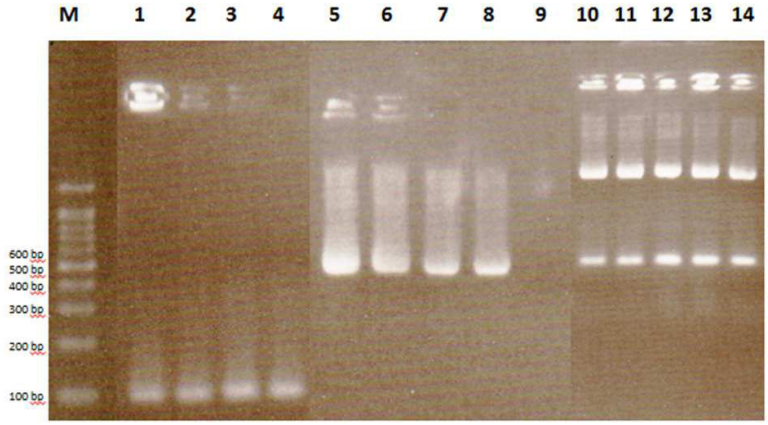

Figure 2

Detection of *S. aureus* specific gene and *mecA* gene by PCR. M, 100-bp molecular weight standard (marker); Lane 1-3; PCR product of three *S. aureus* isolates amplified using *S. aureus* specific gene primer (amplicon size 107 bp); Lane 4 positive *S. aureus* control with specific gene; Lane 5-7, PCR product of three *S. aureus* isolates amplified using *mecA* gene primer (amplicon size 532 bp); Lane 8, positive MRSA control with specific and *mecA* gene; Lane 9, negative control (MSSA) and Lane 10-14, PCR products of five selected *S. aureus* isolates amplified using both *S. aureus* specific gene and *mecA* gene primers

DISCUSSION

In recent years, incidence of multidrug resistance in Gram-positive cocci causing infections in critically ill patients has increased notably. Thus, therapeutic proposals have been modified according to the emergence of multidrug resistant cocci and adapted to epidemiological markers of individual infectious processes, geographical variations of these markers and the availability of new antibacterial agents[13]. Out of 91 patients, 98 bacterial isolates were obtained and the ratio of males (48.4%) and females (51.6%) was recorded. Other investigators have reported a higher proportion of males[14,15, 16]. Centers with a preponderance of males are often military hospitals where service men mainly comprise the patient population. We found a wide range of age groups with a mean of 38.04± 26.68 years is comparable to previous communications from the same hospital a few years back [17]. Distribution of Saudi (41.7%) and non-Saudi (58.2%) was very similar to that reported previously from the same hospital[17]. There appears to be no significance in terms of gender, nationality or age. Staphylococci, enterococci and streptococci were detected most frequently in this study which is in agreement with earlier studies in Makkah hospitals[18]. Most isolates were from the ICUs in particular the frequency CoNS was highest from this unit. This corroborates the well established belief that these organisms are opportunists and their prevalence is highest among patients who are debilitated and pre-disposed to infections[5]. In this study, coagulase-negative staphylococci were most frequently isolated from blood samples. Other studies reporting, a high prevalence of CoNS in blood specimens have commented in detail on the clinical significance of these isolates[19]. During this study it was not possible to establish the importance of CoNS isolates.

The high prevalence of Gram-positive cocci in wound infections is due to the pyogenic nature of these organisms, hence their affinity for such sites[5]. Similar findings have been reported previously[16]. In this study, methicillin-resistant *S. aureus* were most frequently (50%) isolates, obtained from wounds. Other investigators have also reported that most of MRSA isolates originated from skin and soft tissue infections[20]. The proportion of MRSA among all *S. aureus* during this study was 34.5%. This is in close agreement with the percentage of 38% previously reported from the same hospital[17]. Multi-center survey in Kingdom of Saudi Arabia (KSA) reported that, the incidence of MRSA was ranged from 12-49%[16].Encouragingly the prevalence of MRSA in this study was not as high as in another study from this area[21]. Further investigations are needed after an attempt to implement stricter infection control procedures. *Enterococcus faecalis* (72.4%) and *E. faecium* (17.2%) were the most predominant *Enterococcus* species. This is in agreement with previous findings[22,23]. Most strains of enterococci in this study were isolated from surgical unit and ICUs. This corroborated the opportunist nature of these organisms and was in agreement with other studies which indicated the nosocomial nature of these organisms[22,23.] The presence of other co-morbidities has well known to play a role in the prevalence and distribution of resistant pathogen. In particular, previous antibiotic treatment has been shown to play a major role in the incidence of microbial resistance [15, 17]. Our results indicated that Gram-positive cocci were isolated mostly from patients with one or more co-morbidities or previously antibiotic treated (83.5%). In this study all MRSA isolates were resistant to penicillin and ampicillin. This finding was in agreement with other surveys[24]. The percentages of resistance to gentamicin and ciprofloxacin were 40% and 30% respectively which were considerably lower than 75% and 69% previously reported [25.]

Fortunately resistance to vancomycin was not detected during this study which was similarly to that reported by other centers in Saudi Arabia[15,16]. In contrast to this, one report documented a reduce in vancomycin susceptibility in KSA[26] which extremely encouraging particularly as globally differing

levels of vancomycin resistance [27]. In addition, all isolates were sensitive to linezolid which was in agreements with the previously findings[28,29]. Other studies in KSA have reported differing levels of resistance (2-15%) to the previous antibiotic[16]. However, this current finding should not lead to complacency on the contrary strict monitoring and surveillance is warranted. In particular any indication of clinical failure with vancomycin or linezolid should be followed up with stringent laboratory investigations. High incidence of multidrug resistant MRSA strains was reported previously[30] but during this survey, frequency of these organisms was lower (20%). In this study, mupirocin resistance was detected in 20% of the MRSA isolates which was higher than that reported several years back (3%)[14], but is similar to 12% - 28% recently reported from a multi-center study[16]. Other studies have shown that widespread usage of mupirocin in hospitals resulted in increasing plasmid mediated resistance[31]. Incidence of inducible clindamycin resistance was 20% for MRSA and 3.2% for CoNS. This result was lower than that investigated several years back which reported incidence of ICR at 43% for MRSA and 20.7% for CoNS[32]. The sensitivity to clindamycin and erythromycin were performed using Vitek 2 card but this technique was less sensitive as D-zone test[33]. Other investigators have previously reported sensitivity of all *St. agalactiae* strains to a range of antimicrobials commonly used [27] and this finding was confirmed in this study.

During this study, isolates of *E. faecium* were highly resistant to several common antimicrobials such as vancomycin, penicillin, ampicillin, erythromycin and levofloxacin. High levels of resistance have similarly been reported before[23,34]. Resistance to linezolid was not detected in this study. About 10.7% of enterococcal strains were resistant to vancomycin. Nowadays, vancomycin resistant strains have been the subject of considerable debate globally and VRE are becoming increasingly common in many hospitals especially among nosocomial infections[22]. Other investigators have previously reported multiple drug resistance in VRE[34]. Resistance among *E. faecalis* has been documented before but at much lower levels. During this study, *E. faecalis* showed lower levels of resistance to a range of antimicrobials and no resistance to vancomycin or linezolid was detected. The low prevalence of vancomycin resistance among the isolates in this study indicated that vancomycin retains its therapeutic efficacy against the majority of enterococci isolated from patients in Saudi hospitals.Level of resistance to gentamicin and streptomycin were high in *E. faecalis* and *E. faecium* isolates confirming previous reports[23, 35]. This high level of resistance is a cause for concern, as it may signify the beginning of a major resistance problem.Two types of primers were used to amplify *S. aureus* specific gene and *mecA* gene using PCR technique. Fragments of 107 and 532 base pair were detected in all tested isolates of MRSA (100%) from PCR products which were specific for *S. aureus* specific gene and *mecA* gene, respectively. The previous isolates were identified phenotypically as *S. aureus* after bacteriological examination and appeared oxacillin/cefoxitin resistant. Molecular techniques specially *S. aureus* specific gene and *mecA* gene have higher sensitivity, accuracy and specificity for MRSA detection. In addition, they were not time consuming. Similar results were obtained in other studies[36, 37, 38].

CONCLUSION

This study revealed that staphylococci were isolated most frequently followed by enterococci and then streptococci. Most of the isolates were nosocomial acquired from inpatients. Wounds and abscess were the main sources of MSSA and MRSA in ICU patients. Prevalence of MRSA strains among patients was similar to that of a previous study at the same center. Some differences in frequency of MRSA isolates can be a result of different patient population, or different infection control procedures. *Enterococcus faecalis* and *E. faecium* were the predominant species in enterococcal isolates which were more frequently isolated from urine specimen. Incidence of *E. faecalis* was greater in

the surgical unit while prevalence of *E. faecium* was the highest in ICUs. Vancomycin-resistant *E. faecium* was detected from patients in medical units. Incidence of resistant Gram-positive cocci was higher in patients who had other underlying diseases. Resistance to oxacillin and cefoxitin was detected in 34.48% of *S. aureus* isolates. Mupirocin resistance was detected in 20% of the MRSA isolates and 20% of MRSA isolates were multidrug resistant. All MRSA isolates were susceptible to vancomycin, teicoplanin, tigycycline and linezolid.About 10.3% of *Enterococcus* spp. was resistant to vancomycin which indicated that vancomycin retains its therapeutic efficacy against the majority of enterococci. Although no vancomycin resistance among MRSA was observed and low levels detected in enterococci, regular surveillance studies, infection control and monitoring of antibiotic sensitivity among hospital-isolated strains is still required. Molecular technique remains the most sensitive method (100% accurate) in detecting *S. aureus* specific gene and *mecA* gene in MRSA, when compared with the classical methods.

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