

Epidemiology and Antibiotic Resistance of Gram-Positive Cocci Isolated from Infected Swabs and Blood Specimens

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ABSTRACT: Resistance to antimicrobial drugs is a significant health problem affecting the world. Bacitracin A is an efficient growth inhibitor for many Gram-positive bacterial species, but it has little impact on Gram-negative microorganisms. This study aimed to estimate the antibacterial activity of bacitracin against Gram-negative bacterial infections which isolated from clinical specimens. A cross-sectional quantitative study involving bacteriological analysis was conducted at Al Saleem medical laboratory from January 2021 and August 2022. In general, blood, fluid and swabs specimens are collected from the wounds suspected to be infected and were sent for culture to the Microbiology Department. A total of 51 (68.9%) *Staph aureus* isolates was obtained from wound swabs, followed by 5 (6.7%) *Strep pneumonia*. Gender does not appear to have any effect on the presence and prevalence of the different identified gener.. In the present study, Somewhat, Gram-positive bacteria were intermediately susceptible to bacitracin antibiotic. In *staph aureus* isolates, 10.8% were resistant to the Bacitracin antibiotic. 9.4% of *Strep pneumonia* showed intermediate susceptibility to it. Bacitracin is a broad-spectrum antibiotic that can be used to prepare a variety of therapeutic formulations such as infections, wounds and microbiological infections.

KEYWORDS: BACITRACIN, INTERMEDIATE, WOUND SWAB, STAPH AUREUS

I. INTRODUCTION

Resistance to antimicrobial drugs is a major health problem affecting the world. In Libya, misuse of anti-microbial agents by the public is widespread. As in numerous developing countries antimicrobials can be purchased from pharmacies without a prescription in Libya. Wound sepsis continues to be a

significant problem in surgery despite the availability of potent antimicrobial agents. Average rates of wound sepsis are reported to be 5-10 %,4, 5 but vary from 1% to over 50%, 6 depending on the population under consideration and the kind of surgery performed. Device use is recognized as creating a high risk of catheter-associated urinary tract infections (CAUTIs), central line-associated bloodstream infections (CLABSIs), and ventilator-associated pneumonia (VAP) in ICU. 2, 3 Patients. Group A streptococcus, or *Streptococcus pyogenes*, is a facultative, Gram-positive coccus which causes a wide range of diseases in humans, from mild to life-threatening ones, such as pharyngitis, scarlet fever, tonsillitis, cellulitis, impetigo, vulvovaginitis, pneumonia, endocarditis, meningitis, sepsis and necrotizing fasciitis. 1 Antibiotic-resistant Gram-positive bacteria including *Staphylococci*, account for up to 70% of nosocomial infections among patients. Bacitracin is produced by strains of *Bacillus licheniformis*. The commercial product contains the main component, bacitracin A, and at least nine additional closely related polypeptides. 7 In a neutral or slightly alkaline solution, bacitracin A is slowly transformed into bacitracin F, which has very little antibacterial activity. Bacitracin A is highly inhibitory towards the growth of many gram-positive bacterial genera but, has little effect on gram-negative microorganisms. 8 It inhibits bacterial cell wall synthesis and induces the accumulation in *Staphylococcus aureus* of UDP-acetylmuramyl pentapeptide, a precursor of the cell wall. 8 The development of resistance to bacitracin in vitro by serial transfer of bacteria in the presence of sub-inhibitory concentrations of the drug has been studied with *Staphylococcus aureus* and haemolytic streptococci. 8 Resistance arose irregularly during approximately 40 transfers and reverted rapidly when the

resistant strains were subculture in the absence of the drug. 7 Aims of this study were to evaluate the antibacterial activity of Bacitracin against gram-negative bacterial infections isolated from clinical specimens

II. EXPERIMENTAL

A. Study Setting and Design.

A cross-sectional quantitative study involving bacteriological analysis was conducted at Al salem medical laboratory from January 2021 and August 2022.

B. Procedure for Wound Cultures

Generally, samples (blood, fluids, and swabs) were collected from suspected wounds and sent to the Microbiology Department for testing and cultivation. Samples were smeared on clean Gram-stained glass slides and examined for shapes under a microscope. [18] Next step, the samples were arrayed on MacConkey agar and blood agar medium under sterile conditions and incubated aerobically at 37 °C overnight. [19-20] Visual examination of bacterial colonies indicates culture positivity. The suspected organism was then identified by a Gram spot and a series of biochemical tests, including a catalase test, mannitol salt agar with egg yolk and Vitek 2 GP (VITEK 2 Automated ID/AST Device). Strains are identified using procedures recommended by the Clinical and Laboratory Standards Institute (CLSI). Finally, the antibiotic susceptibility pattern was detected using a Vitek 2 AST GP 67 (bioMérieux, Singapore). And conduct susceptibility tests for antimicrobial drugs depending on the isolated organism.

C. Antibiotic susceptibility testing

Antimicrobial susceptibility was first identified by the disk diffusion method on Muller-Hinton agar supplemented with 5% sheep blood using the disk of commonly used antibiotics in Iran and by Clinical and Laboratory Standards Institute (CLSI 2012) guideline. 10 The antibiotic used was Bacitracin

D. Data Analysis

The data was analysed by SPSS programs version 20.

III. RESULTS AND DISCUSSION

A. Distribution of bacteria isolated from patient samples culture by sex.

Sex seems to have no influence on the presence and prevalence of the different identified genera.

TABLE I. Distribution of bacteria isolated from patient samples culture by sex.

Isolates	Gender		Total
	Female	Male	
MRSA	1	1	2
<i>Strep pneumonia</i>	4	5	9
<i>staph aureus</i>	18	39	57
<i>staph epidermidis</i>	2	1	3
<i>strep pyogens</i>	1	0	1
<i>strep viridans</i>	0	2	2
Total	26	48	74

B. Distribution of bacteria isolated from patient samples culture by samples.

More than half of the wound cultures analyzed showed bacterial growth, and this finding is consistent with a study conducted in India, which indicated that 59.8% of wound cultures had bacterial growth.

11 A total of 51 (68.9%) *Staph aureus* isolates were obtained from wound swabs, followed by 5 (6.7%) *Strep pneumonia*. Wound patients were most commonly infected with Gram-positive bacteria, especially *staph aureus* isolates. In other studies, the most common bacteria were *Staphylococcus aureus*. 11, 12 Identifying common bacteria in wound infections can allow for the determination of causative isolates for healthcare providers in remote or resource-limited settings.

TABLE II. Distribution of bacteria isolated from patient samples culture by samples.

Isolates	sample				Total
	Blood	Fluid	Wound swab	Conjunctiva swab	
MRSA	1	0	1	0	2
<i>Strep pneumonia</i>	0	2	5	2	9
<i>staph aureus</i>	4	1	51	1	57
<i>staph epidermidis</i>	1	0	2	0	3
<i>strep pyogens</i>	0	0	0	1	1
<i>strep viridans</i>	0	2	0	0	2
Total	6	5	59	4	74

C. Antimicrobial resistance patterns of isolates to Bacitracin.

The United States FDA permitted the clinical use of bacitracin in 1948 for the treatment and prevention of acute and chronic skin infections. Intramuscular injection of bacitracin can also be given for the systemic treatment of streptococcal pneumonia and emphysema. However, in 2020, the Food and Drug Administration requested that bacitracin be removed from the market. 15 Bacitracin was the only one of the specific inhibitors of bacterial cell wall synthesis that inhibited P-lipid dephosphorylation. Antimicrobial susceptibility determined by both disc diffusion and broth dilution methods. In the current study, Gram-positive bacteria were moderately sensitive to the antibiotic bacitracin. Bacitracin has bactericidal effects as well as affecting Gram-positive bacteria such as *Staphylococcus* spp and *Streptococcus* spp 9

TABLE III. Antimicrobial resistance patterns of isolates to Bacitracin.

Susceptibility patterns	Frequency	Percent
Intermediate (I)	58	78.4
Resistant (R)	9	12.2
Sensitive (S)	7	9.5
Total	74	100.0

D. Antibiotic susceptibility pattern (%) of resistant Gram positive isolates in patient's specimens.

Bacitracin is a polypeptide antibiotic. It inhibits bacterial cell wall synthesis and induces the accumulation in *Staphylococcus aureus* of UDP-acetylmuramyl-Penta peptide, a precursor of the cell wall. 7 The development of resistance to bacitracin in vitro by serial transfer of bacteria in the presence of sub-inhibitory concentrations of the drug has been studied with *Staphylococcus aureus* 16 *Staph aureus* isolates, 10.8% were resistant to Bacitracin antibiotic. Bacitracin is a topical antibiotic used to treat skin injuries such as cuts, scrapes and burns by medical personnel and the general population or by intramuscular injection of bacitracin can also be given for the systemic treatment of *staphylococcal pneumonia* and emphysema. 8 9.4% *Strep pneumonia* showed intermediate susceptible to it.

TABLE IV. Antibiotic susceptibility pattern (%) of resistant Gram positive isolates in patient's specimens.

Isolates	Bacitracin			Total
	I	R	S	
MRSA	1	0	1	2

<i>Strep pneumonia</i>	7	1	1	9
<i>staph aureus</i>	44	8	5	57
<i>staph epidermidis</i>	3	0	0	3
<i>strep pyogens</i>	1	0	0	1
<i>strep viridans</i>	2	0	0	2
Total	58	9	7	74

E. Distribution of infected specimens according to gender.

The wound pus was the most samples from which bacteria were isolated from both gender, followed by blood, and the least isolation was from the Conjunctiva swab.

TABLE V. Distribution of infected specimens according to gender.

Sample	gender		Total
	Female	Male	
Blood	3	3	6
Fluid	0	5	5
Wound swab	21	38	59
Conjunctiva swab	2	2	4
Total	26	48	74

IV. CONCLUSION

Topical antimicrobials have been used successfully to decrease bacterial infections in wounds for decades. Bacitracin is a broad-spectrum antibiotic with a wide range of biological interventions that may be used to prepare a variety of formulations to treat inflammation, wounds and microbiological infections.

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