

Bacteria causing community acquired superficial skin infections in tertiary care hospitals and their antibiotics susceptibility patterns

Aiman Waheed, Irfanullah Afridi*, Attiya Tareen**, Mehran Khan[#], Dawood Khan^{##}

Center of Biotechnology and Microbiology, University of Peshawar.

*Dermatology Ward, Khyber Teaching Hospital, Peshawar.

** Dermatology Department, Fuji Foundation Hospital, Rawalpindi.

[#] Dermatology ward, Khyber Teaching Hospital, Peshawar.

^{##} DHO, Lower Dir.

Abstract

Objective To find out the antibiotic susceptibility pattern of different types of bacteria causing community acquired superficial skin infections and to find the frequency of MRSA in community-acquired primary pyodermas in outpatients visiting the tertiary care hospitals.

Methods Following informed consent, swab samples were collected from infected skin patches and microbiologic examination done. The collected samples were cultured, followed by Gram stain and different bacterial isolates were then identified by conventional biochemical tests, as per Clinical Laboratory Standard Institute (CLSI) guidelines, the modified Kirby Bauer disk diffusion method was used to test the antibiotic sensitivity with standard antibiotic discs. MRSA were detected by using cefoxitin disc and by determining minimum inhibitory concentrations (MIC) of oxacillin. MRSA isolates were further confirmed by targeting *mecA* gene.

Results Overall 205 patients were analyzed with age in range of 1 month to 60 years (with a median age of 12). The ratio between male and female ratio was 1.6:1. A total of 54 (26.3%) cases that displayed no signs of growth. Growth was observed in 151 (73.65%) cases, 142 (94%) cases showed growth for single bacteria while multiple growth was observed in 9 (6%) cases so a total of 158 bacterial isolates were obtained and processed in this study. Among 205 cases, 56 (27.3%) were of folliculitis, 52 (25.36%) of cellulitis, 44 (21.46%) of furunculosis, 42 (20.48%) of impetigo and 11 (5.36%) were of ecthyma. Among 151 positive cases *Staphylococcus aureus* was found in 50 (33.11%) cases, followed by *Pseudomonas aeruginosa* 32 (21.19%), *Escherichia coli* 31 (20.52%), *Streptococci* 24 (15.89%) and *Enterobacter spp.* 21 (13.90%). *S. aureus* was found to be ampicillin- and amoxicillin-resistant in all cases and 20 (40%) were discovered to be Cefoxitin resistant (MRSA).

Conclusion The antibiotic choice for *S. aureus* should be Clindamycin, Doxycycline, and Linezolid. In case of MRSA, Fusidic acid and Vancomycin should be recommended. Gentamicin and Amikacin demonstrated good susceptibility both for Gram positive and Gram negative isolates. Piperacillin-tazobactam and Tigecycline were found most efficient antibiotics and all isolates were susceptible to them.

Key words

Community acquired superficial skin infections, staphylococcus aureus, methicillin resistant staphylococcus aureus (MRSA), pseudomonas aeruginosa, vancomycin.

Introduction

Pyoderma are common clinical problems and are mostly caused by *Streptococcus pyogenes* and

Staphylococcus aureus.¹ Primary pyodermas are ecthyma, furuncle, carbuncle, sycosis barbae, folliculitis, erythrasma, and impetigo. Along climatic conditions, several other factors

likemaln nutrition, poor hygiene, poverty and overcrowding are also related to prevalence of these infections.³ The etiological aspects of primary pyoderma are changed with time and the occurrence of drug resistance strains is a serious health issue. Pyoderma is mostly caused by *S. aureus*, which may be either methicillin-resistant (MRSA) or methicillin-sensitive (MSSA). An important health care associated pathogen is MRSA and most of these are found to be multiple drug resistant.⁴ The β -lactams counting tazobactam, high-end cephalosporins, carbapenams and piperacillin etc. are ineffective against MRSA. The appropriate empirical treatment for these infections becomes acute due to prevalence of MRSA and its resistance towards commonly used antibiotics.⁴

On the basis of current epidemiological trends and continually changing susceptibility patterns, it is necessary to restructure how common superficial pyodermas are treated. This implies current knowledge of the local bacterial flora's sensitivity patterns as well as accurate operational knowledge of the several antibiotics that are currently available. In Pakistan, the scarcity of such data led to prescription of antibiotic that is mostly ridiculous and influenced by the accessibility of medicines, the client's social background, and personal counseling techniques. This results in financial loss to a third world country and is therefore, the leading cause of antibiotic resistance development.⁵ Keeping all these issues in view, it is necessary to define the complete profile of bacteria causing these infections and their susceptibility pattern in different communities across our diverse country. The present study aimed to determine the pathogens implicated

and their pattern of antibiotic sensitivity in patients with commonly acquired superficial pyodermas in the community reporting for treatment to Dermatology Department of Khyber Teaching Hospital (KTH) and Hayatabad Medical Complex (HMC) Peshawar.

Methods

Samples collection The present study was conducted at Department of Dermatology, Medical Teaching Institute Khyber Teaching Hospital (MTI KTH), Medical Teaching Institute Hayatabad Medical Complex (MTI HMC), Microbiology laboratory of Center of Biotechnology and Microbiology (COBAM), University of Peshawar (UOP), Peshawar from July 2019 to October 2019. The demographic and clinical details of the patients were recorded on purposefully designed Proforma. The swab samples were collected from the infected areas of skin using aseptic technique and were immediately transported to the laboratory for microbiological analysis. The swabs were cultured on Blood agar, MacConkey agar and Mannitol Salt agar media plates and were incubated at 37°C for 24 hours. The colony morphology was used to identify the isolates, gram staining technique, biochemical tests including citrate, urease, triple sugar iron, indole, oxidase, coagulase and catalase as per international guidelines.⁶

The patients who presented with community acquired superficial skin infections and reported for the first time to hospitals irrespective of age and gender were included in the study. The patients using antibiotics for cutaneous infection or those having infection spread to deeper skin layer were excluded from the study.

Antibiotic susceptibility testing After identification, antibiotic sensitivity analysis was conducted using Kirby Bauer disc diffusion

Address for correspondence

Dr. Mehran Khan, Assistant professor,
Department of Dermatology,
Khyber Teaching Hospital, Peshawar.
Ph: +92-300-9112944
Email: mkbaabar@yahoo.com

method on Mueller Hinton Agar media. For each bacterial isolate, the suspension was prepared and the turbidity was adjusted to 0.5 McFarland standard and spread on MHA plate.⁷ Antibiotic discs were placed on the agar plates and incubated overnight at 37°C for 24 hours. The standard antibiotics discs (Oxoid) used for Gram positive isolates included Ampicillin (AMP), Amoxicillin (AMC), Trimethoprim+Sulphamethoxazole (SXT), Doxycycline (DO), Clindamycin (DA), Erythromycin (E), Ciprofloxacin (CIP), Linezolid (LZD), Fusidic acid (FD), Aztreonam (AZM), Cefoxitin (FOX), Vancomycin(VA), Amikacin (AK) and Gentamicin (CN). Similarly for Gram negative isolates included Meropenem (MEM), Imipenem (IPM), Tigecycline (TGC), Piperacillin-tazobactam (TZP), Erythromycin (E), Amikacin (AK), Gentamicin (CN), Ceftriaxone (CRO), Cefepime (FEP), Cefotaxime (CTX), Ceftazidime (CAZ), Cefixime (CFM), Fosfomycin (FOS), Levofloxacin (LEV) and Ciprofloxacin (CIP). After incubation, the diameter of zones were measured for each drug and were categorized as resistant, intermediate and sensitive on the basis of criteria published by Clinical laboratory standard Institute (CLSI).⁸

MRSA detection Methicillin resistance in *S. aureus* was assessed using Clinical and Laboratory Standards Institute (CLSI) criteria based on recommendations utilizing a Cefoxitin (30 g) disc on a swab-inoculated Mueller Hinton Agar (MHA) plate and incubated at a temperature of 37° C for 24 hours.⁹ For all strains of MRSA, the Minimum Inhibitory concentration (MIC) of oxacillin was determined by broth microdilution method as described by Andrews¹⁰ and CLSI M07-A9 guidelines.¹¹ The results were interpreted according to CLSI guidelines.¹² *S. aureus* strain ATCC 25923 was used for the purpose of quality control. Further the MRSA isolates were confirmed by targeting

mecA gene through conventional PCR technique. The PCR was first standardized to detect the suitable annealing temperature for the set of primers used for *mecA* detection: F-CTCAGGTACTGCTATCCACC, R-CACTTGGTATATCTTCACC with amplicon size of 449 bp.^{12,13} The conditions of amplification were: initial denaturation at 94°C for 30 seconds, followed by 30 cycles of DNA denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 30 seconds. The final extension was done at 72°C for 7 minutes. The amplification was done in thermocycler. The reaction mixture for PCR was prepared by mixing 11.5 µL of Nuclease-free water, 12.5 µL of Taq master mix (Bioron, Life sciences), (Fermentas#R0581), 2 µL of sample DNA and 1.5 µL of each forward and reverse primers (Macrogen, Oligonucleotides, Korea). For positive control *S. aureus* strain ATCC 33591 was used while negative control, only PCR mixtures with no DNA template was used. Analysis of amplified PCR product was done by electrophoresis (Bioron, Life sciences) on 2% agarose gel.

Results

Overall 205 patients were analyzed with age in range of 1 month to 60 years (with a median age of 12). The ratio of male and female was 1.6:1. Among the collected 205 cases, 56 (27.3%) cases were of folliculitis, 52 (25.4%) of cellulitis, 44 (21.46%) of furunculosis, 42 (20.48%) of impetigo and 11 (5.36%) cases were of ecthyma (**Table 1**). The lower extremities were involved in 45% cases followed by head (29%), upper extremities (13.5%), and trunk (12.5%) respectively. There were 54 (26.34%) cases that did not reveal growth of any microorganism. Among 151 positive cases, 142 (94%) showed growth of single isolate and remaining 9 (6%) showed multiple growth, as a result, 158 bacterial strains in total were isolated

Table 1 Community-acquired superficial skin infections on the basis of number and form of microorganisms

Microorganisms	Number of isolates (%)					
	Cellulitis (n=43)	Folliculitis (n=38)	Furunculosis (n=32)	Impetigo (n=28)	Ecthyma (n=10)	Total (n=158)
Staphylococcus aureus	8 (18.6)	19 (50)	9 (28.1)	10 (35.7)	4 (40)	50(31.7)
Pseudomonas aeruginosa	14 (32.6)	7 (18.4)	5 (15.6)	6 (21.4)	—	32(20.25)
Escherichia coli	13 (30.2)	3 (7.9)	9 (28.1)	4 (14.3)	2 (20)	31(19.62)
Streptococcus	6 (14)	6 (15.7)	8 (25)	2 (7.1)	2 (20)	24(15.18)
Enterobacter spp	4 (9.3)	4 (10.5)	3 (9.4)	8 (28.6)	2 (20)	21(13.3)

Table 2 Antimicrobial sensitivity profile of isolates from routine superficial skin disorders.

Antibiotics	Gram positive isolates		Antibiotics	Gram negative isolates		
	<i>S. aureus</i> (n=50)	<i>Streptococcus</i> (n=24)		<i>P. aeruginosa</i> (n=32)	<i>E. coli</i> (n=31)	<i>Enterobacter spp</i> (n=21)
AMP	1	8	MEM	28	29	18
AMC	2	14	IPM	29	30	19
SXT	16	18	TGC	32	31	21
DO	34	20	TZP	32	31	21
DA	33	19	E	10	14	8
E	25	15	AK	21	23	13
CIP	23	17	CN	17	19	16
LZD	30	20	CRO	16	17	13
FD	45	24	FEP	12	14	8
AZM	32	22	CTX	19	16	8
FOX	30	24	CAZ	16	18	6
VA	50	24	CFM	18	19	12
AK	36	23	FOS	13	15	9
CN	38	24	LEV	20	18	8
			CIP	3	6	2

(Table 1). *S. aureus* was the most commonly isolated bacteria followed by *P. aeruginosa*, *E. coli*, *Streptococci* and *Enterobacter spp.* (Table 1). Antibiotics susceptibility results of all the clinical isolates are mentioned in Table 2. All the *S. aureus* were found resistant to Ampicillin and Amoxicillin and were found susceptible to almost all other antibiotics tested. Forty percent of *S. aureus* were resistant to Cefoxitin and were marked as MRSA. The MRSA were found resistant to almost all antibiotics tested. Vancomycin was found most effective drug followed by fusidic acid, Gentamicin, Amikacin, Doxycycline, and Clindamycin were all effective against all isolates. Streptococci were found susceptible to almost all the antibiotics except Ampicillin. In case of Gram negative isolates, Piperacillin-tazobactam and Tigecycline were found most efficient drugs as all the isolates were found susceptible to them followed

by Meropenem, Imipenem, Amikacin and Gentamicin. Methicillin resistance is generated by the *MecA* gene in Gram-positive bacteria, particularly *S. aureus*. In our study, MIC driven testing and disc diffusion identified 20 *S. aureus* (40%) as MRSA. All methicillin resistance *S. aureus* were tested for identification of *Mec A* gene. The current study shows that *Mec A* genes were found in the entire sample as shown in Figure 1.

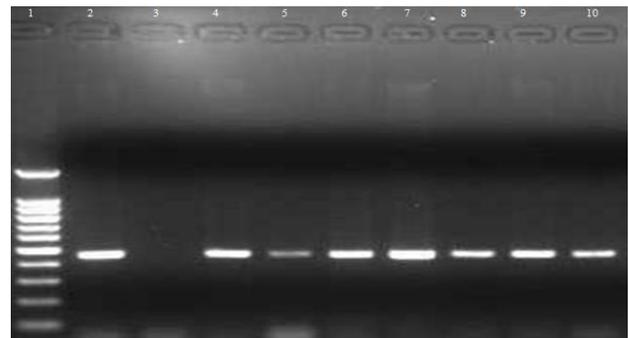


Figure 1 *mecA* gene detection.

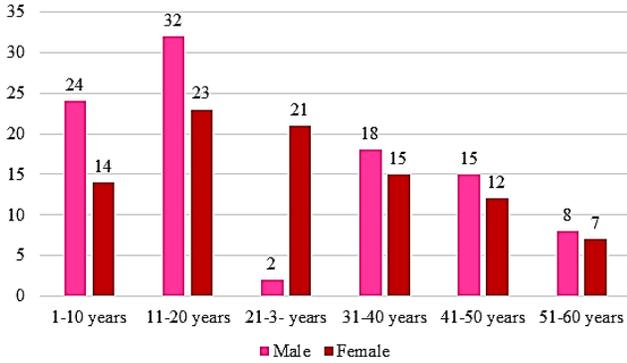


Figure 2 Age and gender distribution.

Discussion

Community acquired pyodermas are global problem and affect people of all ages. Group A streptococci and *S. aureus* are most commonly isolated microorganisms often occurring as primary pyodermas. Secondary pyodermas raise concerns about gram-negative infections and mixed infections.¹⁴ In some international and local studies, the increased prevalence of MRSA had been observed.¹⁴

In the present study, the most commonly found infections were folliculitis, cellulitis and furunculosis, seen in 27.3%, 25.4 and 21.48 of cases respectively. In some other studies, folliculitis and furunculosis were the most frequently detected primary pyodermas.^{15,16} Bhaskaran *et al.* also found superficial folliculitis (25.9%) to be the most prevalent followed by impetigo (16.36%) and furunculosis (4%).¹⁷ In contrast to our findings, Ghadage DP *et al.* found impetigo (39%) to be the most common pyoderma followed by superficial folliculitis (13%), and carbuncle (1.5%), respectively.¹⁸ Mathew *et al.* also concluded the same findings.¹⁹ Although in one of studies, impetigo was the commonest lesion in children.²⁰ However, most of the patients in our study were adults.

Growth was observed in 151 (73.66%) of samples and 54 (26.34%) samples showed no

growth. In one study, 14.9% cases were found as culture negative by Baslas *et al.*¹⁶ 142 (94%) samples showed growth for single bacteria while multiple growths was observed in 9 (6%) samples. Polymicrobial infections in range of 5-16% were also observed in other studies.²⁰

In our study 45% cases presented with lower limbs followed by head (29%), upper limbs (13.5%), and trunk (12.5%), similar results were also found in previous studies.^{19,20}

Similar to previous studies, the dual etiology of organisms in impetigo was also found in our study.¹ The isolation rate of isolates differs significantly in the present study but similar to some studies in cases of impetigo, the predominant organism is *S. aureus*.^{14,20-22} Gram positive bacteria were found to be the primary pathogens followed by Gram negative as secondary pathogens. Alternatively *S. aureus* is major causative agent instead of Streptococcus pyogenes in furunculosis/ folliculitis. This finding is analogous to the outcomes of previous studies.^{23,24}

It is noted in this study that in case of cellulitis *P. aeruginosa* and *E. coli* were predominant isolates as compared to *S. aureus*. This finding is in contrast to other studies which reported streptococcal as predominant etiology of cellulitis.¹

In this research, *S. aureus* 50 (31.7%) growth was shown by culture followed *P. aeruginosa* 32 (20.25%), *E. coli* 31 (19.62%), β -hemolytic *Streptococci* 24 (15.18%) and *Enterobacter spp.* 21 (13.3%). Similar to our study, 48.6% prevalence of *S. aureus* was recorded by Bhaskaran *et al.*,¹⁷ 67% by Ghadage *et al.* and 97% in study of Parikh *et al.*^{18,26} In addition to our study, *S. aureus* was found as most prevalent isolate in previous studies.¹⁴⁻¹⁶

Some of *S. pyogenes* were found susceptible to β lactamase sensitive penicillins while most of the isolates were resistant to them. Consequently, considering the causative organism of superficial pyodermas, these penicillins would not be practical for empiric usage. Maximum of *S. aureus* were found sensitive to lincosamides (lincomycin and clindamycin), fusidic acid, Amikacin, gentamicin, or doxycycline so they might be initiated as empirical topical and/or systemic treatments and enhanced in accordance with the findings obtained in terms of culture and sensitivity.

In present study, 40% cases of *S. aureus* were detected as MRSA while remaining 60% were MSSA. Nagaraju *et al.* from Mangalore found that 11.8% *S. aureus* strains isolated from pyodermas were methicillin resistant.¹⁴ Agreeing to the National Staphylococcal Phage Typing Centre, New Delhi, a rise in the prevalence of MRSA strains was recorded from 9.83% in 1992 to 45.44% in 1998.²⁷ The prevalence of MRSA in community acquired pyoderma in study of Nagaraja *et al.* from India was 10.9%.²⁸ However, isolation rate of MRSA from pus samples was 83% in study of Qureshi *et al.* from Pakistan.²⁹ The predominance of MRSA in the community and in community-acquired pyodermas have been emphasized by many studies from Asia and India.^{14,20,30,31}

In accordance with our study the susceptibility rates of Gram negative isolates and MRSA to doxycycline and erythromycin do not make them a suitable empirical choice against them. Other studies also observed low susceptibility towards erythromycin.¹⁴ As compared to Rennie *et al.* study, in our study some of the samples were found resistant to co-trimoxazole, and cephalosporins,²³ therefore these should not be selected as a drug of choice for empirical treatment in cases of superficial pyodermas. Chloramphenicol was found as most efficient

antibiotic in our study majority of gram positive isolates were found susceptible to it so it must be used in treatment of pyodermas and can also be selected as drug of choice for empirical treatment especially for topical purpose.

The isolation of MRSA (40%) from our cases differs from cases in other studies.¹⁴ However this finding is comparable with studies from hospital acquired pyodermas.²³⁻²⁵ Majority of the methicillin resistance *S. aureus* were found sensitive to commonly used antibiotics like lincosamides, chloramphenicol and ciprofloxacin. MRSA strains have appeared as severe nosocomial pathogens throughout the last few years and because of its ability to become resistant to different antibiotics, its spread worldwide acquire resistance to antimicrobial chemotherapy.^{32,33} So, speedy recognition of such organisms and methicillin resistance detection are essential for stimulating effective treatment, stopping spread of these pathogens and decreasing the patient's mortality risk.^{34,35}

Similar to previous study, our study also found that *mecA*-positive *Staphylococci* showed low susceptibility rate to the antibiotics tested as compared to *mecA*-negative^{34,36} and also had proved that MRSA carry resistance genes to many different antibiotics.

Conclusion

Globally, microbiology laboratories should maintain a watchful eye on locally prevalent resistant infections, and they should routinely report MRSA, ESBLs, and MBLs. Pyoderma treatment should be based on the results of an antibiogram. The inability of isolates to be further validated by genotypic techniques due to a lack of resources was a limitation of this work. Understanding the local resistance mechanisms of pathogens and implementing tactics to stop the spread of such illnesses in a hospital are both

made possible by phenotypic detection.

Primary superficial pyodermas are among commonly occurring clinical problem dermatological setting. Initial choice of antibiotic for *S. aureus* should be Clindamycin, Doxycycline, and Linezolid. In case of MRSA, Fusidic acid and Vancomycin should be recommended as topical/ systematic substitute and for decisive management, culture & sensitivity of suitable sample would be obligatory after wards. However, most of causative agents were found susceptible to chloramphenicol so it can be used for empirical treatment. Gentamicin and amikacin were both effective against gram positive and gram negative isolates. Pipiracillin-tazobactam and tigecycline were found most efficient antibiotics and all isolates were susceptible to them.

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