Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 570-576

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.068

A Study of Bacterial Isolates from Impetigo and their Resistance Pattern to Mupirocin

B. Cinthujah[1], G. Sucilathangam[2*], A. Balakumaran[1] and C. Revathy[1]

[1]Department of Microbiology, Tirunelveli Medical College, Tirunelveli - 627 011, Tamil Nadu, India
[2]Department of Microbiology, Government Theni Medical College, Theni - 625512, Tamil Nadu, India

**Corresponding author*

Keywords

Impetigo, Methicillin resistant *Staphylococcus aureus* (MRSA), Antibiotic susceptibility testing, Mupirocin

Article Info

Accepted:
04 August 2019
Available Online:
10 September 2019

A B S T R A C T

Impetigo continues to be an important paediatric skin infection. *Staphylococcus aureus* is the commonest bacteria associated with Impetigo. Drug resistance particularly to first line oral antibiotics is alarmingly high in *Staphylococcus aureus.* This study aims to find the bacterial isolates causing impetigo and their susceptibility pattern to drugs particularly Mupirocin. This prospective study was done in patients presenting with impetigo to the dermatology OP of a tertiary care hospital for the period of six months from May 2017 to October 2017. All the samples were collected aseptically with two sterile cotton swabs for each sample from the lesion, which were processed for isolation and identification of bacterial pathogens, according to the standard microbiological techniques. The prevalence was common in the Paediatric age group. Scabies was commonly associated with impetigo in 13%. *Staphylococcus aureus* was the cause in 63 % of impetigo followed by *Streptococcus pyogenes* in 3% of patients. *Streptococcus pyogenes* strains still remain susceptible to all the common antibiotics. Methicillin resistant *Staphylococcus aureus* (MRSA) was found in 41.3% of the 63 *Staphylococcus aureus* strains. The prevalence of High level Mupirocin in the community was 0% and Low level Mupirocin resistance was 4.76%. Mupirocin is one of the commonest drugs used for topical use in Impetigo and is effective against both *staphylococcus and streptococcus* and not effective against the normal cutaneous flora. Mupirocin is also used to eradicate nasal and cutaneous carriage of *Staphylococcus aureus*.

Introduction

Impetigo is a highly contagious skin infection affecting the epidermis. It commonly affects school going children with a case load of more than 10 million cases in India. It clinically manifests as honey coloured scabs formed from dried serum (Oumeish *et al.,* 2000). It is caused by infections with the Gram positive cocci *Staphylococcus aureus and Streptococcus pyogenes* (Brown *et al.,* 2003). Infection usually heals without leaving a scar unless the dermis is involved (ecthyma) and the rare case of cellulitis. But *Streptococcal* infection carries a risk of post streptococcal glomerulonephritis.

Topical antibiotics are the treatment of choice for most cases. Treatment reduces the spread of the bacterial strains through contact and fomites, and that of the rare nephritogenic strain. Mupirocin is one of the commonest drug used for topical use in Impetigo and is effective against both *staphylococcus and streptococcus* and not effective against the normal cutaneous flora (Booth and Benrimoj, 1992).

Mupirocin is also used to eradicate nasal and cutaneous carriage of *Staphylococcus aureus*. But bacteria particularly *Staphylococcus* have developed resistance to Mupirocin (Booth and Benrimoj, 1992) .

Two types of resistance against Mupirocin are seen with *Staphylococci;* the low level resistance strains showing resistance to 5µg disc but susceptible to the 200µg Mupirocin disc and the high level resistance strains showing resistance to both the 5µg and the 200 µg disc (Jean *et al.,* 2009; Oommen *et al.,* 2010). This study aims to find the bacterial isolates causing impetigo and their susceptibility pattern to drugs particularly Mupirocin.

Materials and Methods

After approval by the Institutional Scientific and Ethics Committee this prospective study was done in patients presenting with impetigo to the dermatology OP of a tertiary care hospital for the period of six months from May 2017 to October 2017.

Study population

A total of hundred non repetitive swabs were collected from patients presenting with impetigo to the dermatology OP of a tertiary care hospital after consent. Comprehensive history was taken along with physical and dermatological examination for all the patients.

Inclusion criteria

1) Patients with skin lesion suggestive of Impetigo. 2) Patients not admitted to hospital at present or for a period of up to one year before.

Exclusion criteria

1) Patients with lesion with pus characteristic of other pyoderma. 2) Patients who were hospitalized at present or at any time for a period of up to one year before.

Sample collection

All the samples were collected aseptically with two sterile cotton swabs for each sample from the lesion, which were processed for isolation and identification of bacterial pathogens, according to the standard microbiological techniques. Gram stain preparations were made from one swab, and culture plates were inoculated from another swab. Each sample was inoculated on blood agar, MacConkey agar, and Mannitol salt agar. The media were prepared according to the manufacturers' instructions. The plates were incubated at 37°C for 18-24 hours in an incubator.

The plates were observed for growth the following day but incubation was extended to 48 hours if there was no bacterial growth within 24 hours. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Identification was carried out according to the standard biochemical tests.

Staphylococcus aureus was confirmed using Gram staining, Cataiase test, growth on Mannitol salt agar, Coagulase test and sensitivity to Furazolidone. *Streptococus* was confirmed by the typical beta haemolytic colony on blood agar plate, a negative catalase test and sensitivity to Bacitracin.Anti-

microbial susceptibility test was carried out on isolated and identified colonies using commercially prepared antibiotic disc (HiMedia) on Mueller Hinton agar plates for *Staphylococcus aureus* and on blood agar for *Steptococcus pyogenes* by the disk diffusion method, according to the Central Laboratory Standards Institute (CLSI) guidelines.

Antibiotics used in this study were Amoxicillin (100 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg),Clindamycin(2 µg) Vancomycin (30 µg) Mupirocin (5µg) and Mupirocin (200 µg).

The plates were incubated for 24 hours at 35°C, and zone diameter was measured. MRSA detection was done using Cefoxitin disc (30µg). A zone size of greater than 22mm was considered to be sensitive and the *S. aureus* isolate described Methicillin sensitive.

Mupirocin Susceptibility testing was done using both 5 and 200µg Mupirocin discs. A zone diameter of greater than 14 mm for both 5 and 200 µg discs were considered to be susceptible to Mupirocin. Isolates that showed zone diameter less than 14mm for the 5µg disc but more than or equal to 14 mm for the 200 µg disc were considered low level resistant and the isolates showing zone diameter less than 14 mm for both 5 and 200 µg were considered Mupirocin high resistant strains.

D- test for determination of inducible Clindamycin resistance was done on all isolates while doing drug susceptibility testing Erythromycin disc was placed at a distance of 20 millimetre to the Clindamycin disc. After overnight incubation, the plates were observed for flattening of zone of inhibition of the Clindamycin disc adjacent to the Erythromycin disc which indicates Inducible Clindamycin resistance. They are reported as Clindamycin resistant.

Results and Discussion

A total of one hundred (100) patients with impetigo attending the dermatology OP were studied by detailed history and clinical examination. Swabs were collected and were subjected to Gram staining and bacteriological culture. The individual bacterial isolates and their sensitivity pattern to various antibiotics were recorded and analysed.

Out of the hundred patients with impetigo fifty eight patients belonged to the paediatric age group below 14 years (58%). There were forty two patients in the Adult category (42%). The ratio of male to female is 63:37. In this study out of the 100 non duplicate swabs sixty six samples grew pathogens (66%). There was no growth or growth of commensal bacteria in thirty four samples (34%). Out of the sixty six bacterial isolates that grew sixty three were *S. aureus* (63%) and three were *S. pyogenes* (3%) (Table 1).

Out of the 100 samples a total of thirteen patients had impetigo associated with scabies.(13%). Of the sixty three *S. aureus* isolates all the sixty three were resistant to Ampicillin (100%) and there was no resistance to high level Mupirocin and Vancomycin (0%). Twenty six isolates of *S. aureus* showed resistance to Methicillin (41.3%). Eighteen isolates of *S. aureus* showed resistance to Clindamycin (28.6%). Twenty nine isolates of *S. aureus* showed resistance to Erythromycin (46%). Three isolates of *S. aureus* showed resistance to low level Mupirocin (4.8%). All the three isolates of *S.pyogenes* showed no resistance to the panel of antibiotics tested (0%) (Table 2).

Twenty six isolates of *S. aureus* showed resistance to Methicillin (41.3%). Eighteen isolates of *S. aureus* showed resistance to Clindamycin (28.6%). Twenty nine isolates of *S. aureus* showed resistance to Erythromycin

(46%). Three isolates of *S. aureus* showed resistance to low level Mupirocin (4.8%). All the three isolates of *S. pyogenes* showed no resistance to the panel of antibiotics tested (0%).

Table.1 Sex-wise distribution of Bacterial isolates

ORGANISMS	MALE	FEMALE	TOTAL
Staphylococcus aureus	36	27	63(63%)
Streptococcus pyogenes	3	0	3(3%)
No growth/commensals	24	10	34(34%)

Table.2 Antibiotic sensitivity and resistance pattern of Bacterial isolates

Antibiotic	*Staphylococcus aureus* N=63		*Streptococcus pyogenes* N=3	
	Sensitive	Resistance	Sensitive	Resistance
Ampicillin	0 (0%)	63 (100%)	3 (100%)	0 (0%)
Mupirocin (5µg)	60 (95.24%)	3 (4.76%)	3 (100%)	0 (0%)
Mupirocin (200µg)	63 (100%)	0 (0%)	3 (100%)	0 (0%)
Clindamycin	45 (71.42%)	18 (28.58%)	3 (100%)	0 (0%)
Erythromycin	34 (53.96%)	29 (46.04%)	3 (100%)	0 (0%)
Cefoxitin	37 (58.73%)	26 (41.27%)	Not tested	Not tested
Vancomycin	63 (100%)	0 (0%)	3 (100%)	0 (0%)

In the present study prevalence of impetigo in children was 58%. This correlates with the studies of Manju mohan *et al.*, and Shashi Gandhi *et al.*, who also had a high percentage of impetigo infections in children (Manju mohan *et al.*, 2016; Shashi Gandhi *et al.*, 2012). Close personal contact with friends and family members, increased physical trauma due to high physical activity outdoors and low level of hygienic practices are the reasons for increased infection in paediatric group. Environmental factors like overcrowding and malnutrition also play a role in children from low socio economic groups.

In this study there was an increased incidence of impetigo seen among males (63%). This correlates with the study of Shashi Gandhi and Manju Mohan (Manju mohan *et al.*, 2016; Shashi Gandhi *et al.*, 2012). This can be attributed to the increased time spent by males

doing physical activity outdoors which increases the risk of trauma. Scabies was present in 13% of cases with impetigo in this study. This corresponds with the studies of Asha C. Bowen *et al.*, where 17% of children with impetigo had scabies (Asha C. Bowen *et al.*, 2014). The children were mostly of Australian aborigine community and were of low socio economic status. This present study also is in line with Shashi Gandhi *et al.*, who found scabies in 8% of cases with impetigo (7). This can be explained by the fact that Scabies is essentially a disease of children and as it produces intense itching which predisposes to trauma leading to bacterial infection. In fact Impetigo or other pyodermas at sites of scabies predilection is one of the commonest clinical manifestation of scabies. The scabies infections associated with *Streptococcal* pyodermas is one of the important predisposing factors for nephritis in the paediatric age group.

In the current study, out of 100 cases of Impetigo 63% grew *Staphylococcus aureus* and 3% grew *Streptococcus pyogenes.* This correlates with the study of Justin brown who also found that impetigo infections due to *S. aureus* are becoming more common than *Streptococcus.* This is also similar to the study by Shashi Gandhi *et al.*, who had *S. aureus* in 86 (81%) and only 5 (5%) beta haemolytic *streptococcus* among 106 impetigo cultures whereas Asha C. Bowen *et al.*, had 81% *S. aureus* infections and 44% *Streptococcus pyogenes* (Asha C. Bowen *et al.*, 2014; Daniel K. Yeoh *et al.*, 2017). Co-infection with both bacteria was common (37%) in their study. In this study 35 samples had no growth or grew *Micrococci* or *Diphtheroids* or aerobic spore bearers. This could be due to minimal number of pathogens due to prior oral or topical antibiotics.

There was a contact history with impetigo cases in 32% of the cases. There was a clustering of impetigo cases in families with all the siblings infected. This validates the name impetigo contagiosa given to non-bullous impetigo. The choice of antibiotic discs was Ampicillin, Cefoxitin as a surrogate marker for Methicillin resistance), Erythromycin, Clindamycin, Vancomycin and Mupirocin (5µg and 200 µg). Ciprofloxacin and Doxycycline were avoided as they are contra indicated in paediatric age group who form a large population in our study. We could not get a double strength Co-trimoxazole disc which is recommended as a treatment for MRSA.

All the 3 *Streptococcus pyogenes* showed no resistance to Ampicillin, Erythromycin, Clindamycin, Mupirocin and Vancomycin (0%). In this study, all the isolates of *Staphylococcus aureus* were resistant to Ampicillin (100%). This is of concern because Amoxicillin is the empirical antibiotic prescribed to patients with impetigo in our hospital along with Mupirocin 2% cream.

Injudicious use of this antibiotic has lead to this miserable condition. The prevalence of MRSA in the present study is 41.3%. As all our isolates were collected from OP patients they are representative of CA-MRSA. In the present study higher percentage of antibiotic resistance to Erythromycin, Clindamycin and low level Mupirocin was found among the MRSA strains. This findings correlate with that of Yeoh *et al.*, who had a 39% prevalence of MRSA (Daniel K. Yeoh *et al.*, 2017). In their study Manjumohan *et al.*, had 19.1% MRSA strains (Manjumohan *et al.*, 2016).

The high degree of resistance to other antibiotics in MRSA strains also reported by Nagaraju *et al.*, study (Nagaraju *et al.*, 2004). It is disturbing to see high resistance to both erythromycin (46%) and Clindamycin (28.6%) among community *Staphylococcus*

aureus strains. D test was positive in 28.6% of samples. This implies the presence of inducible resistance to Clindamycin and extrapolates to resistance to all MLSB antibiotics.

In this study among MRSA strains 69.23% were resistant to Erythromycin and 30.76% were resistant to Clindamycin. Daniel K Yeoh *et al.,* in their study of hospitalized children found that 23% of strains of MRSA were resistant to Clindamycin (Daniel K. Yeoh *et al.,* 2017). This high degree of antibiotic resistance can be attributed to the easy availability of antibiotics over the counter and poor people getting medications from medical shops and unqualified self-styled medical personnel which is common in India. They fail to complete the entire course prescribed for that antibiotic which leads to résistance. Three (4.76%) cases of low level Mupirocin resistance. In at least in one among these three cases there was long term irregular use of Mupirocin ointment. The patient's impetigo later progressed to ecthyma with local lymphadenopathy and was treated with hospital admission and systemic antibiotics. She had varicose veins also. All the three isolates were also Methicillin resistance strains.

No case of high level resistance was noted. Its better not to have high level Mupirocin resistance as bacteria carrying the plasmids coding for this type of resistance is easily transmissible. Oommem *et al.,* in their study found no low level Mupirocin resistance in both MRSA and MSSA strains but 2.08% of MRSA strains showed high level Mupirocin resistance and 1.02% of MSSA strains showed high level Mupirocin resistance whereas Rudresh *et al.,* found low level Mupirocin resistance in 17% and high level Mupirocin resistance in 8.2% *Staphylococcus aureus* strains (Oommem *et al.,* 2010; Rudresh *et al.,* 2015).

There are several studies in which high level Mupirocin resistance in *S. aureus* is associated with Mupirocin in treatment and decolonization failure. The association between low level Mupirocin resistance and the outcome of Mupirocin decolonization is not clear. But it is generally accepted that low level Mupirocin resistant strains still be treated with Mupirocin because MIC of low level Mupirocin resistant was greater than 4µg/ml but less than 512µg/ml which is the cut off level for high level Mupirocin resistant. But 2% Mupirocin cream obtains a concentration of 20,000 µg/ml in the skin after 24 -36hours of exposure.

The newer classes of topical antibacterials like Mupirocin, sodium Fusidate are not freely available in different parts of India and even if available are not affordable by people from low socioeconomic strata, who are the common sufferers of skin and soft tissue infections (Oommem *et al.,* 2010).

The reason for absence of high level Mupirocin resistance in this study could be attributed to fact that only recently (for the past one year) Mupirocin ointment is available in this hospital's pharmacy and the high cost of the drug at medical stores which limits its use. It is confounded by the study of Jean *et al.,* that as the rate of usage increases resistance to Mupirocin increases.(Jean *et al.,* 2009) They site a great variation of 63% Mupirocin resistance in a hospital where there was high usage for nasal decolonization and 6% resistance in another hospital where it was rarely used.

At present Mupirocin ointment is not used for skin decolonization of MRSA carriers. It is used as a topical treatment for impetigo. May be it is time to devise a protocol on which patients to use Mupirocin and whom to use topical antiseptics like Cetrimide. Mupirocin may be used if there was a failure of treatment

with topical antiseptics or those who are immunocompromised like diabetes etc. We are in danger of losing an effective and safe choice if wise decisions are not made and indiscriminate usage continues.

In conclusion, impetigo continues to be an important paediatric skin infection and association with scabies is significant. Drug resistance particularly to first line oral antibiotics is alarmingly high in *Staphylococcus aureus*. This warrants judicious use of antibiotics and drug susceptibility testing in centres where resources are available. There is an urgent need to device an antibiotic policy which is applicable to all levels of the health care system.

Acknowledgement

The authors gratefully acknowledge ICMR, New Delhi, The Dean, Tirunelveli Medical College Hospital, Tirunelveli, Tamil Nadu and The Staff of Dermatology and Microbiology, Tirunelveli Medical College Hospital.

References

Asha , C . Bowen., Stephen, Y. C. Tong and Mark, D. Chatfield. (2014). The microbiology of impetigo in Indigenous children association between *Streptococcus pyogenes Staphylococcus aureus* scabies and nasal carriage. BMC infectious disease, 14: 727.

Booth, J. H., Benrimoj, S. J. (1992). Mupirocin in the treatment of impetigo. Int J Dermatology: 31: 1-9.

Brown, J Shriner., Schwartz, N., A., Janniger, C. K. (2003) Impetigo an update. Int J Dermatology, 42: 251-255.

Daniel, K. Yeoh., Aleisha Anderson, G.C., Asha, C. Bowen. (2017) Are scabies and impetigo "normalized"? A cross sectional comparative study of hospitalized children in northern Australia assessing clinical recognition and treatment of skin infections. PLOS Neglected tropical diseases V 11 (7).

Jean, B. Patel., Rachel, J. Gorwitz., John, A.J. (2009). Mupirocin resistance. Clinical infectious diseases, 49: 935-941.

Manjumohan, E.N., Abdul latheef, K., Sarada Devi. (2016) Clinico bacteriological study of pyodermas in a tertiary care centre in south India, 82(5): 532-534.

Nagaraju, U., Bhat, G., Kuruvila, M.Pai, Jeyalakshmi, G.S., Babu, R.P. (2004) Methicillin resistant *Staphylococcus aureus* in community acquired pyoderma. Int journal of Dermatology, 43: 412-4.

Oommen, S.K., Appalaraju, B., Jinsha, K. (2010) Mupirocin resistance in clinical isolates of staphylococci in a tertiary care centre in south India. Indian J of Medical Microbiology, 28(4): 372-5.

Oumeish, I., Oumeish, O., Y., Bataineh, O. (2000) Acute bacterial skin infection in children. Clinical dermatology, 18; 667-678.

Rudresh, M.S., Ravi, G.S., Aravind, M., Ann Mary, A.P., Navaneeth, B.V.(2015) Prevalence of Mupirocin Resistance Among Staphylococci, its Clinical Significance and Relationship to Clinical Use J Lab Physicians, Jul-Dec; 7(2): 103–107.

ShashiGandhi, A.K., Ojha, K.P. Ranjan. (2012) Clinical and bacteriological aspects of pyoderma. North American Journal of Medical Sciences, 4(10): 492-495.

How to cite this article:

Cinthujah, B., G. Sucilathangam, A. Balakumaran and Revathy, C. 2019. A Study of Bacterial Isolates from Impetigo and their Resistance Pattern to Mupirocin. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 570-576. doi: https://doi.org/10.20546/ijcmas.2019.809.068