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Original Research Article

Mupirocin Resistance among Clinical Isolates of Staphylococcus Aureus from Skin and Soft Tissue Infection in Tertiary Care Centre, Tamilnadu

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Abstract:

Staphylococcus aureus is the most common pathogen responsible for skin and soft tissue infections (SSTIs) worldwide. Mupirocin (pseudomonic acid A) has been widely used as a topical antimicrobial agent for treating SSTIs caused by Staphylococcus aureus. It is an analogue of isoleucyl adenylate and binds to the bacterial isoleucyl-tRNA synthetase (IleS) target near ATP-binding sub-site. Most isolates that demonstrate high-level mupirocin resistance have acquired plasmid-mediated mupA, which encodes a novel isoleucyl RNA synthetase. Mupirocin resistance is being reported in many parts of the world. In India, mupirocin resistance (25%) among MRSA was reported. Hence, this study was undertaken to screen for mupirocin resistance among Staphylococcus aureus isolates. A non-repetitive S. aureus isolates were obtained from patient with SSTI were processed by standard procedures. Antibiotics tested included, Cefoxitin (30 µg) disc for MRSA screening, Mupirocin (200µg) disc used to distinguish isolates with high-level resistance from isolates that are either susceptible or have low-level resistance, Mupirocin (5µg) disc used to distinguish isolates that are susceptible to mupirocin from isolates with either low-level or high-level. The PCR was carried out for the detection of ileS2 (mupA) gene responsible for the high- level mupirocin resistance. A total of 96 isolates included in this study, of which 76 (79%) were found to be MRSA and 20 (21%) isolates were found to be MSSA. Of 96 S. aureus isolates 11% were found to be mupirocin resistant, of which 7(63%) found to carries mupA gene and all of them were MRSA, suggesting there might exist the possibility that high level resistant strains evolve in this study area and also to screen for both low level mupirocin resistance and high level mupirocin resistance by disc diffusion for better appropriate results.

Keywords: SSTI, High-level mupirocin resistance, Low-level mupirocin resistance and MRSA.

Introduction

Staphylococcus aureus is the most common pathogen responsible for skin and soft tissue infections (SSTIs) worldwide. Mupirocin (pseudomonic acid A) has been widely used as a topical antibiotic for treatment of impetigo and secondary wound infection caused by S. aureus and Streptococcus pyogenes. It is an analogue of isoleucyl adenylate and binds to the bacterial isoleucyl-tRNA synthetase (IleS) target near ATP-binding sub-site. Mupirocin resistance is being reported in many parts of the world viz- Spain 11.3%, USA 13.2%, Trinidad Tobago 26.1%, China 6.6%, India 6%, Turkey 45% and Korea 5%. A recent study from India, mupirocin resistance (25%) among MRSA was reported [3].

Three categories of mupirocin susceptibility have been described for S. aureus by two disc strategy (5µg and 200µg disc), are mupirocin susceptible (>14mm Zone around both 5 and 200µg disc), Low- level mupirocin resistance (>14mm zone around 5 µg and no zone around 200µg disc) and High-level mupirocin resistance (No Zone around 5 µg and No zone around 200µg disc). Most isolates that demonstrate high-level mupirocin resistance have acquired plasmid-mediated mupA, which encodes a novel isoleucyl RNA synthetase.

Isolates with low-level mupirocin resistance usually have acquired base changes in the native isoleucyl RNA synthetase gene, ileS [1]. Isolates with low-level mupirocin resistance but positive for the mupA gene have been identified. In these isolates, the mupA gene was located on the chromosome rather than on a plasmid. Also, isolates that are mupA positive by PCR but mupirocin susceptible have also been reported. This has been attributed to a frameshift mutation in the mupA gene that inactivates the gene product. A few isolates have been identified that demonstrate high-level mupirocin resistance but are mupA negative by PCR despite the use of multiple primer

sets. These isolates may carry a novel mechanism of mupirocin resistance[1]. A novel mechanism of highlevel mupirocin resistance mediated by mupB gene has been recently identified [2]. Hence, the present study was undertaken to screen for mupirocin resistance among Staphylococcus aureus isolates from clinical specimens of skin and soft tissue infection from tertiary care centre, Dharmapuri district of Tamil Nadu, South India.

Material and Methods

A prospective study was conducted from the period of December 2018 to September 2019 in Tertiary care hospitals, Dharmapuri. The institutional Review Board on Human Ethics, Government Dharmapuri Medical College, reviewed the study design, protocol and approved the study (GDMCH/Human Ethical Com/Micro/17/11/2018). Non repetitive S. aureus isolates were obtained from patient with SSTIs and were processed by standard procedures.[4] For all phenotypic methods, S. aureus ATCC 25923 was used as control.

Antimicrobial drugs tested included, cefoxitin (30 µg) for MRSA screening, mupirocin (200µg) disc used to distinguish isolates with high-level resistance from isolates that are either susceptible or have low-level resistance, mupirocin (5µg) disc used to distinguish isolates that are susceptible to mupirocin from isolates with either low-level or high-level resistance[5] (Table.1) and interpreted as per CLSI guidelines[6]. Molecular detection of high-level mupirocin resistance was done by using simple PCR method[7]. The primers amplify ileS2 (mupA) gene responsible for the high- level mupirocin resistance.

The PCR was carried out in 25µl reaction volume containing 1X PCR buffer, 200µM of each dNTP, 1U taq DNA polymerase, primers (10pmol each) and 5ng of template DNA. Amplification was done using Eppendorf Gradient 94°C/1min; annealing 57°C/30s; extension 72°C/1min, final extension of 72°C/ 5minutes and hold at 4°C. The amplified PCR products were separated by agarose gel electrophoresis (1.5% low melting agarose with 0.5% ethidium bromide; 0.5X TBE buffer at 150V and 90mA for 30 minutes){Medox India pvt Ltd}. The bands were visualized and recorded using gel documentation system (BIO-RAD).

Results

A total of 96 clinical isolates of S aureus collected in the period of December 2018 to September 2019 from Govt. Dharmapuri Medical College and hospital, Dharmapuri, were included in this study. Of the 96 clinical isolates, 76 (79%) were found to be MRSA and 20 (21%) isolates were found to be MSSA.

Table 1: Phenotypic categories of S aureus isolates by Mupirocin -2 disc strategy

Sl No	Phenotypes	*AST by Mupirocin 5µg disc	*AST by Mupirocin 200µg disc
1	Susceptible to Mupirocin	Zone >14mm	Zone >14mm
2	Low level resistance to Mupirocin	No zone	Zone >14mm
3	High level resistance to Mupirocin	No zone	No zone

*AST- Antibiotic sensitivity test

Out of 96 isolates of S aureus, 11(11%) were found to be resistant to mupirocin, of which 9(82%) isolates were found to be high level mupirocin resistant and 2(18%) were found to be low level mupirocin resistant strains. Among 11 mupirocin resistant isolates, 8(73%) isolates were MRSA and 3(27%) were MSSA(Table.2).

Table: 2. Mupirocin susceptibility of MRSA and MSSA by disc diffusion test

Mupirocin disc	Phenotypes	MRSA(76)	MSSA(20)
5µg disc	S	68	17
	R	8	3
200 µg disc	S	68	17
	LMR	1	1
	HMR	7	2

S-susceptible; R-resistant;LMR-low level mupirocin resistant; HMR-high level mupirocin resistant

Among 11 mupirocin resistant S.aureus isolates, 7(64%) only found to carries mupA gene and all of them were high-level mupirocin resistant –MRSA (Plate.1). All the isolates with low level mupirocin resistance was not found to carries mupA gene, similarly 18.5% of isolates with high level mupirocin resistance not carries mupA gene.

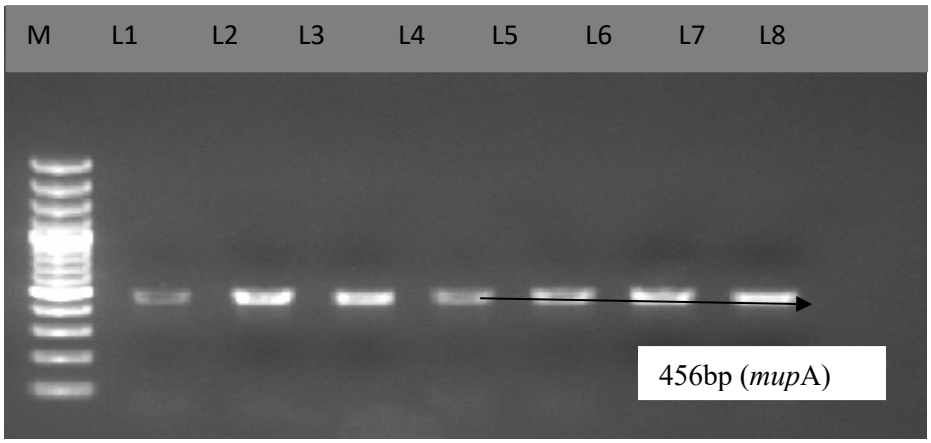

Figure 1: PCR for the detection of mupA gene (Yun et al., 2003)

L1 to L8- mupA; L8- Negative; M- 100 base pair ladder

Discussion

Mupirocin resistance is being reported in many parts of the world, higher rate in Turkey (45%) and low in Korea (5%)[8]. In this study, 11(11%) of S. aureus isolates were found to be mupirocin resistant, of which 9(82%) isolates were showed high level resistance. However, various studies have reported higher percentage of S. aureus isolates were resistant to mupirocin, in India 19.6% [3] in China 17.6% [9]. MRSA showed higher resistance to mupirocin (73%) than MSSA (27%) in this study, it is comparable with a report of Sanju et al., (2015)[3]. Among 11 mupirocin resistant S.aureus isolates, 7(63%) found to carries mupA gene and all of them were MRSA. On contrary, Oommen et al., (2010)[10] have reported 2% of MRSA isolates were high level mupirocin resistant.

A majority of high level mupirocin resistant strains were MRSA isolates in this study, strongly associated with mupA gene, suggesting there might exist the possibility that high level resistant strains evolve in this area. There are several studies in which, high-level mupirocin resistance in S. aureus is associated with mupirocin decolonization failure[1].

While screening for mupirocin resistance better to screen for both low level mupirocin resistance and high level mupirocin resistance by disc diffusion or E test for better appropriate results. Further study is needed to screen for mupB gene to rule out novel mechanism of mupirocin resistance among mupirocin resistant S aureus isolates.

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