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ORIGINAL ARTICLE

Phage typing, PCR amplification for *mecA* gene, and antibiotic resistance patterns as epidemiologic markers in nosocomial outbreaks of methicillin resistant *Staphylococcus aureus*

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Abstract *Staphylococcus aureus* is one of the major causes of community and hospital-acquired infections. Bacteriophage considered as a major risk factor acquires *S. aureus* new virulence genetic elements. A total number of 119 *S. aureus* isolated from different specimens obtained from (RKH) were distinguished by susceptibility to 19 antimicrobial agents, phage typing, and PCR amplification for *mecA* gene. All of MRSA isolates harbored *mecA* gene, except three unique isolates. The predominant phage group is belonging to the (mixed group). Phage group (II) considered as an epidemiological marker correlated to β-lactamase hyper producer isolates. MRSA isolates indicated high prevalence of phage group (II) with highly increase for phage types (Ø3A), which were correlated to the skin. Phage types (Ø80/Ø81) played an important roll in Community Acquired Methicillin Resistant *S. aureus* (CAMRSA). Three outpatients MRSA isolates had low multiresistance against Bacitracin (Ba) and Fusidic acid (FD), considered as CAMRSA isolates. It was detected that group **I** typed all FD-resistant MSSA isolates. Phage groups (M) and (II) were found almost to be integrated for Gentamycin (GN) resistance especially phage type (Ø95) which relatively increased up to 20% in MRSA. Tetracycline (TE) resistant isolates typed by groups (II) and (III) in MSSA. Only one isolate resistant to Sulphamethoxazole/Trimethoprim (SXT) was typed by (III/V) alone in MSSA. MRSA isolates resistant to Chloramphenicol (C) and Ba were typed by all groups except (V). It could be concluded that (PERSA) *S. aureus* isolates from the wound that originated and colonized, and started to build up multi-resistance against the topical treatment

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antibiotics. In this study, some unique sporadic isolates for both MRSA and MSSA could be used as biological, molecular and epidemiological markers such as prospective tools.

1. Introduction

Staphylococcus aureus is a versatile important human pathogen causing a number of variety medical infections. In the other wise, the fact that 90% of hospital staff are carriers of *S. aureus* portends serious for the epidemiology and pathogenesis of Staphylococcal infections (Fey et al., 2003; Lacey et al., 1984). The wide spread of antibiotic resistance among *S. aureus* strains is a major concern in the treatment of Staphylococcus infections. These strains often resistance to multiple antibiotics with attendant increased morbidity; the surveillance and control of these strains was highly desirable (Mathur and Mehudiratt, 2000; Lyon and SKurray, 1987). The increasing importance of MRSA as a cause of nosocomial infections can be inferred from several recent studies (Schmitz et al., 1997). There is relatively little information on the diversity of strains causing infection. It is important to have knowledge of the most common strains associated with human infections and their sources in each episodes and environment in order to improve our understanding of the epidemiology of this pathogen and solve the problems. Although bacterial interaction is a well recognized phenomenon, there has been surprisingly little research with respect to MRSA and MSSA. The mechanism/s responsible for this phenomenon is not readily apparent. Gopal Rao and Wong (2003) concluded that there is a complex relationship between various strains of EMRSA and MSSA especially in the skin. This interaction may have an important bearing on colonization of patients with MRSA. It may explain some of the epidemiological and clinical observations as well as understanding the methods for the movement of resistant genes, like: Transduction (phages) – Plasmids – Integrons – Transposons.

In Saudi Arabia, significant increase of MRSA was noticed from 6.9% to 33% starting in the last decade from 1995 up to 2004 alarming it remarkably, as an outbreak of EMRSA and EMSSA. Several investigations were done concerning the screening and emergence of MRSA in the Kingdom. Belkum et al. (1997) identified that MRSA Saudi isolates all belonging to phage group III. It was clear that consensus *SmaI* pattern observed for Saudi strains was different from other non-related isolates.

Kishan et al. (1998) reported for the most β-lactamase-producing isolates of *S. aureus* belonging to other phage groups. Skov et al. (1995) have reported that all phage group II isolates recovered prior to harbored *blaz* on the chromosome. The final event in multiplication of phage was lysis of the host cell by murein hydrolyses of various substrate specificities (Young, 1992; Loessner et al., 1995, 1998).

The genetic studies that showed that drug-resistance genes were easily transduced among *S. aureus* cells by prophage (Mitsuhashi et al., 1965). This suggested a relationship between the intracellular state of the drug-resistance genes and temperate phages. Thus, they could show that some of the temperate phages transduced the drug-resistance genes.

The aim of this study is to approach questions related to the spread of MRSA in Saudi cohort by examining phenotypic (resistance to antibiotics, phage typing) and the existed genetic backgrounds *mecA* gene. As well as defined the epidemic drift of phage-types within originated susceptible wild type *S. aureus* population as a microbial biomarkers for monitoring the usage of antibiotics. And analyze the contribution of the phage typing, as a phenotypic marker and answer question such as: are the phage types between isolates similarly or differently?

- What is the homogeneity and heterogeneity between MSSA and MRSA?
- Which of *S. aureus* isolates carrying the *mecA* gene?
- What are the antimicrobial resistance determinants that phage could contribute?

2. Materials and methods

2.1. Bacterial isolates

A total numbers of 119 isolates of *S. aureus* from different patients were collected over a period of one year from 2003 to 2004 from microbiology laboratory in Riyadh Armed Forces Hospital (RAFH). The following reference strains of bacterial species were used as controls:

Methicillin resistant *S. aureus* (MRSA) (NCTC 10442), methicillin sensitive *S. aureus* (MSSA) (ATCC 25923)
Coagulase Negative *Staphylococcus epidermidis* (CNS) (ATCC 12228).
Identification of *S. aureus* isolates

The suspected colonies were identified according to the following criteria:

2.2. Colonial morphology

All isolates were streaked for purity growth on Blood Agar plates (Blood Agar Base.Oxoid.Code: CM55) for over night incubated at 37 °C (Collee et al., 1989).

2.3. Microscopic examination

Gram stain smears from a Staph Culture showed gram positive cocci in clusters.

2.4. Catalase test

This test detects the presence of cytochrome oxidase enzymes in *Micrococcaceae* according to Konerman et al. (1992).

2.5. Coagulase test

Slide agglutination test (Staphurex) based on the detection of clumping factor and protein A. The test was performed by Slidex Staph-kit (BioMereux, Chabonnieres-Bain France) according to MacFaddin (1980) and Baron et al. (1994).