Pharmaceutica
Analytica Acta

Malki Fatima et al., Pharm Anal Acta 2012, S15
http://dx.doi.org/10.4172/2153-2435.S15-003

Research Article Open Access

Identification and Characterization of *Staphylococcus* Isolates in Fes-Meknes Region in Morocco

EL Malki Fatima[1], EL Lekhlifi Zineb[2] and Barrijal Said[2]*

[1]*Institut Pasteur du Maroc of Tangier, Morocco*
[2]*Faculty of Sciences and Techniques, Tangier, Morocco*

Abstract

Staphylococci are among the most commonly recovered bacteria in the clinical microbiology laboratories. According to the coagulase test, staphylococci are categorized as *S. aureus* and coagulase negative staphylococci (CoNS). Although *S. aureus* is more virulent, CoNS may also cause infections, some of which may be life-threatening. Raising staphylococci multi-drogue resistance has complicated therapeutic protocols especially for *S. aureus* resistant to meticillin (SARM). In our study we characterized by phenotypic and molecular data, a group of staphylococci collected from hospital laboratories. Comparison between standard laboratories procedures in routine identification and molecular methods showed a good correlation of both. Multiplex-PCR revealed that all species specific 16S RNA positive were coagulase positive and identified to be *S. aureus*. Besides, all *mecA* positive isolates were cefoxitin resistant. The prevalence of SARM was found 11.76% while meticillin resistant coagulase negative staphylococcus (MRCoNS) was 84.61%.

Although the analyzed staphylococcal isolates sample is small, our study revealed a relatively low prevalence of SARM in this region of Morocco compared to others region of the world. The very high prevalence of MRCoNS isolates is alarming and demonstrating that CoNS are becoming the main source for dissemination of *mecA* gene.

Keywords: *Staphylococcus aureus*; CoNS; SARM; Multiplex-PCR; *mecA*

Introduction

Staphylococcus genus currently consists of 38 species [1]. *S. aureus* is the most important pathogen and the most virulent wile *S. epidermidis* and other coagulase negative *Staphylococci* (CoNS) are commensally common flora of skin and mucous membranes and are therefore common contaminants, particularly in blood cultures. However, increased incidence of infections caused by CoNS has been reported [2]. These bacteria have also become an important cause of hospital-acquired infections [3]. Discrimination between *S. aureus* and others CoNS in clinical samples is important to evaluate the virulence level. Moreover, rising *Staphylococcus* isolates multi-resistant to antibiotics has complicated the antimicrobial therapeutic scheme.

Hospital infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) strains are frequent worldwide [4]. In fact, MRSA is considered as a serious threat to hospitalized patients and is becoming a challenge for public health. Moreover, MRSA community-acquired infections appear to be on the increase in various part of the world [5]. The consequences to the health care system are graves with longer hospitalization, greater costs in addition to higher mortality and morbidity rates due to invasive MRSA infection [6].

Thus, rapid and reliable detection and identification tests is an important infection control policy method and might help the clinician to reduce the use of inappropriate therapy, preventing the dissemination of this microorganism.

In Morocco few data are available concerning the MRSA frequency in hospitals or community. This study aims to assess the MRSA frequency in the central region of Morocco comparing the standard phenotypic methods and PCR method to differentiate *S. aureus* from CoNS.

Material and Methods

Bacterial isolates

One hundred forty one isolates, among them sixty two *Staphylococcus,* were collected from hospital bacteriological laboratories in Fes-Meknes region during a period of three months (1st July to 30th September 2010).

Identification of *staphylococcal* isolates

The isolates were identified morphologically and biochemically by standard laboratory procedures. The coagulase plasma test was performed on organisms exhibiting typical *Staphylococcal* colony morphology to allow discrimination of *S. aureus* from coagulate-negative *Staphylococci* [7].

Staphylococcus antibiotic susceptibility testing

Resistance to β-lactams (including methicillin) is assessed by Cefoxitin disk (FOX 30 µg). In addition, susceptibility to fourteen other antibiotics were determined by the agar disk diffusion method. Antibiotics tested are listed: cefoxitin (FOX), vancomycin (VA), teicoplanin (TEC), amikacin (AN), tobramycin (TM), netilmecin (NET), erythromycin (E), lincomycin (L), tertacyclin (TE), chloramphenicol (C), ciprofloxacin (CIP), rifampecin (RA), corticomoxasol (SXT), fosfomycin (FOS), fusidic acid (FA) [8].

***Corresponding author:** Barrijal Said, Faculty of Sciences and Techniques, Tangier, Morocco, E-mail: barrijal@yahoo.fr

Received March 16, 2012; **Accepted** March 29, 2012; **Published** March 31, 2012

Citation: Malki Fatima EL, Lekhlifi Zineb EL, Said B (2012) Identification and Characterization of *Staphylococcus* Isolates in Fes-Meknes Region in Morocco. Pharm Anal Acta S15. doi:10.4172/2153-2435.S15-003

DNA extraction and polymerase chain reaction (PCR)

Staphylococcal colonies were emulsified in 100 µl sterile distilled water to produce a heavy suspension, and heated at 100°C for 15 min, then centrifuged at 12.000 rpm for 10 min.

Multiplex-PCR was performed to detect *mecA* gene and *S. aureus* 16S RNA using the reported primers [9].

Result and Discussion

Bacterial isolates and identification

During a period of three months (from July to September 2010) 241 isolates were collected from hospital laboratories of Fes–Meknes region and routinely identified by standard laboratory procedures. We focused our analysis on *Staphylococcus* group as they occupy the second place, just after *Enterobacteriaceae* with more than 25%, in routinely isolated bacteria from different infection diseases (Table 1 and Figure 1).

Isolates	no	%
E. coli	97	40.24
Staphylocoques	62	25.73
Klebsiella	40	16.60
Pseudomonas	11	4.56
Streptocoque	8	3.32
Acinetobacter	5	2.13
Proteus	5	2.07
Enterobacter	4	1.66
Enterocoque	3	1.24
Bacillus	1	0.41
Providencia	1	0.41
Listeria	1	0.41
Salmonella typhi	1	0.41
Citrobacter	1	0.41
Morganella	1	0.41
Total	241	100

Table 1: Percentage of different isolates.

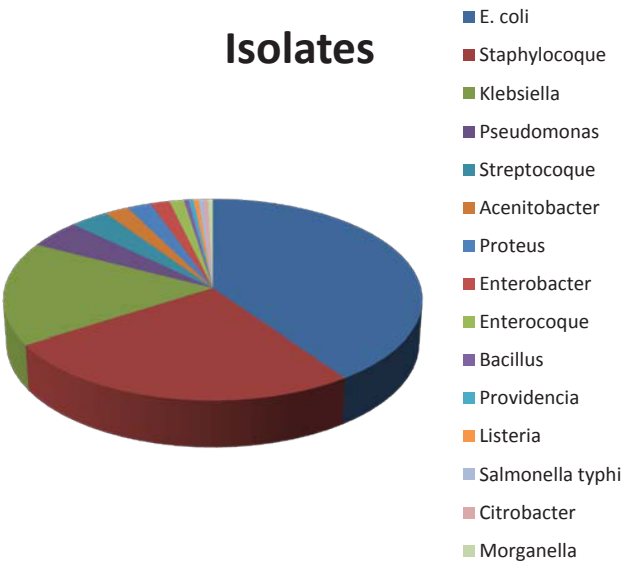

Figure 1: Representation of total collected bacterial isolates.

Samples	no	%
Pus	22	35.48
Urinary	19	30.64
Blood	10	16.13
Urethral	4	6.45
Vaginal	1	1.61
Undetermined	6	9.68
Total	62	100

Table 2: Percentage of samples.

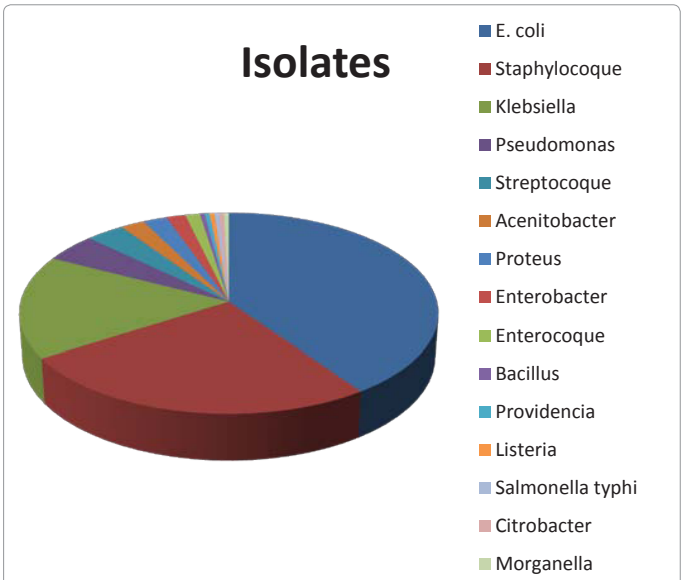

Figure 2: Representation of total collected bacterial isolates.

Distribution of staphylococcal samples

We examined sixty two gram-positive bacteria isolated from different type of human physiological liquid. As showed in table 2, *Staphylococcus* bacteria can be isolated from various kinds of human fluid demonstrating its ubiquitous localization. The pus secretions are the most affected (35.48%) followed by urinary samples (30.64%) shown in Figure 2.

Antibiotic susceptibility

Fifteen different antibiotics were tested. *Staphylococcus* isolates susceptibility rates are showed in table 3. Resistance to cefoxitin is over 36%. The higher rate is detected for tetracyclin (46%) followed by erythromycin (40%) and fusidic acid (33%). The lower resistance rate was reported for amikacin and chloramphenicol (3%) wile lincomycin exhibited 100% susceptibility. For vancomycin and teicoplanin apparent resistance was detected by agar disc diffusion method. The CMI test for vancomycin has been carried out, and these isolate were classed vancomycin susceptible with CMIs of 1 mg/L or 0.5 mg/L. revealing their sensitivity (Table 4).

Antibiotics	R		I	S	
	Nbr	%	Nbr	Nbr	%
FOX	11	36.67	1	18	60
VA	6	20	5	19	63.33
TEC	8	26.67	6	16	30
AN	1	3.33	0	29	96.67
TM	6	20	0	24	80
NET	1	3.33	0	29	96.67
E	12	40	0	18	60
L	0	0	0	30	100
TE	14	46.67	0	16	30
C	1	3.33	0	29	96.67
CIP	9	30	0	21	70
RA	6	20	0	24	80
SXT	5	16.67	0	25	83.33
FOS	5	16.67	2	23	76.67
FA	10	33.33	0	20	66.67

Table 3: Antibiotics susceptibility of *Staphylococci* isolated.

Tableau: Resultat de CMI										
Isolat []	**64**	**32**	**16**	**8**	**4**	**2**	**1**	**0.5**	**0.25**	**0**
61Z	-	-	-	-	-	-	-	+	+	+
92Z	-	-	-	-	-	-	-	+	+	+
97Z	-	-	-	-	-	-	+	+	+	+
101Z	-	-	-	-	-	-	+	+	+	+
103Z	-	-	-	-	-	-	-	+	+	+
90M	-	-	-	-	-	-	-	+	+	+
93M	-	-	-	-	-	-	-	+	+	+
98M	-	-	-	-	-	-	-	+	+	+
99M	-	-	-	-	-	-	-	+	+	+

Table 4: CMI Determination for vancomycin.

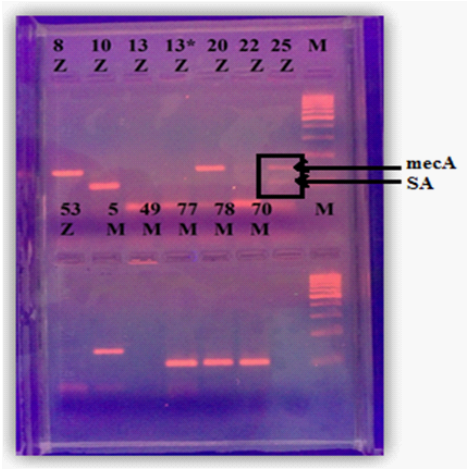

Figure 3: Agarose gel electrophoresis of amplified DNA corresponding to the *S. aureus* species specific segment (SA = 108 bp) and the *mecA* gene (154 bp) detected by the multiplex PCR.

PCR detection of *mecA* gene and 16S RNA

A sub-site of 30 *Staphylococcus* isolates were selected on the basis of their antibiotic resistance profiles to be further characterized by multiplex-PCR to assess the presence of *mecA* gene and *S. aureus* 16S-RNA to confirm the MRSA strains. A sample of multiplex-PCR products are shown by Figure 3. The two genes are detected in 25 isolates and which is then a MRSA. Others isolates presenting only one segment are *mecA* positive (case of meticillin resistant CoNS) or *S. aureus* meticillin sensitive (SAMS).

Conclusion

The obtained results were compared with coagulase test and the cefoxitin susceptibility. These two tests are used in routine procedures to identify MRSA.

Among the 30 *Staphylococcus* 17 were coagulase positive and then considered as *S. aureus* strain and the remained 13 isolates were belonging to CoNS group. Eleven isolates were resistant to cefoxitin ($\Phi \leq 24$ mm) and one was an intermediary phenotype ($\Phi = 25$ mm).

S. aureus 16S RNA was detected by PCR in 17 isolates which is in correlation with the coagulase test [10,11]. *mecA* gene was detected in 13 isolates only two among them showed *S. aureus* species specific segment, and then are MRSA strains. Hence, the frequency of *mecA* gene in *S.aureus* estimated at 11.76% is relatively low (2/17) compared to CoNS group which showed very high prevalence with 84.61% (11/13). The CoNS isolates are becoming the main source for dissemination of *mecA* gene.

Although the analyzed *Staphylococcal* isolates sample is small, our study revealed a relatively low prevalence of SARM in this region of Morocco compared to others region of the world [12].

References

1. Kleeman KT, Bannerman TL, Kloos WE (1993) Species distribution of coagulase-negative staphylococcal isolates at a community hospital and implications for selection of staphylococcal identification procedures. J Clin Microbiol 31: 1318-1321.
2. Edwards KJ, Kaufmann ME, Saunders NA (2001) Rapid and accurate identification of coagulase-negative staphylococci by real-time PCR. J Clin Microbiol 39: 3047-3051.
3. Mekontso-Dessap A, Kirsch M, Vermes E, Brun-Buisson C, Loisance D, et al. (2002) Nosocomial infections occurring during receipt of circulatory support with the paracorporeal ventricular assist system. Clin Infect Dis 35: 1308-1315.
4. Oliveira DC, Tomasz A, de Lencastre H (2002) Secrets of success of a human pathogen: Molecular evolution of pandemic clones of meticillin-resistant Staphylococcus aureus. Lancet Infect Dis 2: 180-189.
5. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, et al. (2003) Community-acquired methicillin–resistant Staphylococcus aureus carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis 9: 978–984.
6. Lodise TP, McKinnon PS (2005) Clinical and economic impact of methicillin resistance in patients with Staphylococcus aureus bacteremia. Diagn Microbiol Infect Dis 52: 113-122.
7. Murray PR (2003) Manual of clinical microbiology. (8thedn), American Society for Microbiology Press, Washington, DC, USA.
8. Lalitha MK (2004) Manual on Antimicrobial Susceptibility Testing (Under the auspices of Indian Association of Medical Microbiologists).
9. Schuenck RP, Lourenco MC, Iório NL, Ferreira AL, Nouér SA, et al. (2006) Improved and rapid detection of methicillin-resistant Staphylococcus aureus nasal carriage using selective broth and multiplex PCR. Res Microbiol 157: 971–975.
10. Jones RN, Nilius AM, Akinlade BK, Deshpande LM, Notario GF (2007) Molecular characterization of Staphylococcus aureus isolates from a 2005 clinical trial of uncomplicated skin and skin structure infections. Antimicrob Agents Chemother 51: 3381-3384.
11. Maltezou HC, Giamarellou H (2006) community-acquired methicillin-resistant Staphylococcus aureus infections. Int J Antimicrob Agents 27: 87–96.
12. Krishnan PU, Miles K, Shetty N (2002) Detection of methicillin and mupirocin resistance in Staphylococcus aureus isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA. J Clin Pathol 55: 745-748.