WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 237-242. Research Article ISSN 2277– 7105

PREVALENCE OF *CFR* GENES AMONG METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN BAGHDAD

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Article Received on
06 Sep 2015,

Revised on 27 Sep 2015,
Accepted on 18 Oct 2015

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ABSTRACT

The aim of this study was to determine the distribution of *cfr* gene in Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are leading causes of hospital-acquired infections in Baghdad. The resistance patterns of MRSA isolates were determined, 23% of MRSA isolates were resistant to chloramphenicol, all isolates were resistant to cloxacillin, followed by cefoxitin (86 %) and cephalexin (51%), 26% to lincomycin and 22% to rifampicin. A total of 23 chloramphenicol resistant MRSA isolates were tested for the presence of the *cfr* gene by PCR, the results showed that *cfr* gene found in 34.7 % of isolates.

KEYWORDS: *cfr* gene, *Staphylococcus aureus*, PCR.

INTRODUCTION

Staphylococcus aureus is an important human pathogen capable of causing diseases in the hospital and community settings. The increased incidence of multidrug-resistant *S. aureus* strains among nosocomial (or hospital-acquired (HA) infections has added a challenging dimension to the *S. aureus* problem.[1,2]

The sharp emergence and spread of methicillin resistant *S. aureus* (MRSA) in the community setting and the occurrence of vancomycin-resistant staphylococci, along with vancomycin-intermediate *S. aureus* (VISA) and the most important heterogeneous VISA, are of concern.[3] This phenomenon has led to the development of new antimicrobial compounds.[4] The *cfr* gene was detected in 1999 on the multiresistance plasmid pSCFS1 in a bovine *Staphylococcus sciuri* strain. Initially, this gene was described as a chloramphenicol–florfenicol resistance gene. Studies of the cfr-mediated resistance mechanism showed that *cfr*

codes for an rRNA methyltransferase that targets the adenine residue A2503 in 23S rRNA, and thereby confers not only phenicol resistance, but also clindamycin resistance.[5] The *cfr* gene encodes an RNA methyltransferase and thereby confers combined resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins and streptogramin A antibiotics (otherwise known as the PhLOPSA phenotype).[6,7]

recent studies showed that cfr was also present in other Gram-positive bacteria such as Bacillus spp., and *Enterococcus faecalis*, from pigs.[8,9] Moreover, *cfr* has also been found in single isolates of Gram-negative bacteria, such as *Proteus vulgaris* and *Escherichia coli*.[10,11] The aim of this study was to determine the frequency of *cfr* genes in MRSA in cutaneous infections in Baghdad.

MATERIALS AND METHODS

Bacterial isolates: A total of 100 MRSA isolates were collected from cutaneous samples (abscess and wound) from patients who were admitted to Baghdad hospitals in 2013.These isolates were identified by conventional biochemical reactions according to the criteria established by Forbes et al.[12] The isolates were inoculated a CHROMagar MRSA plate. The results were read after 24 and 48 h of incubation at 35°C.

Antimicrobial susceptibility test

Antimicrobial susceptibility of the isolates were tested by using Kirby-Bauer disk diffusion method following CLSI guidelines[13], using commercially available 6mm discs (Bioanalyse /Turkey) The susceptibility of the isolates was determined against 13 antibacterial agents, They include: clindamycin ,rifampicin, chloramphenicol, tecoplanine, trimethoprime, cloxacillin, gentamicin, cephalexine, cefoxitine, lincomycin, levofloxacin, Azithromycin and vancomycin, on Mueller Hinton agar Plate(Lab M Limited Topley House, United Kingdom),using overnight culture at a 0.5 McFarland standard followed by incubation at 35 ^{o}c for 16 to 18 h. Moreover, the isolates were tested for their *in vitro* susceptibilities to chloramphenicol by the broth macrodilution method.

DNA Preparation and PCR: The presence of *cfr* gene in MRSA isolates was determined by PCR as described earlier.[14] PCR condition was started the process with initial denaturation step at 94 C/1min was followed by 35 cycles of amplification with denaturation at 94 C for 1 min, annealing at 55 C for 1 min, and extension at 72 C for 3 min, with a final extension at 72

C for 7 min. PCR product was resolved on a 1.5 % agarose gel stained with ethidium bromide and visualized under UV transillumination.

RESULTS AND DISSCUSION

In this research 100 Methicillin resistant *S. aureus* isolates were collected from hospitalized patients in Baghdad. The resistance patterns of MRSA isolates to antimicrobial agents are shown in Table 1. All isolates were resistant to cloxacillin, followed by cefoxitin (86 %) and cephalexin (51 %), 26% to lincomycin, 23% to chloramphenicol and 22% to rifampicin. Other resistance rates were: 18 % gentamicin, 17% vancomycin, while the minimum resistance were seen with teicoplanin (4%).

Table 1. Susceptibility of the 100 isolates of Methicillin-resistant *S.aureus* to Antibiotics.

Antibiotic		Resistance %
Chloramphenicol	C	23
Clindamycin	DA	13
lincomycin	L	26
Levofloxacin	LEV	13
trimethoprime	TEM	23
cloxacillin	CX	100
Azithromycin	AZM	16
cefoxitine	CX	86
gentamicin	CN	18
cephalexine	CL	51
tecoplanine	TEC	4
rifampicin	RA	22
vancomycin	VA	17

In study by Fayyaz et al.[15], out of the 174 MRSA isolates, 38 isolates (21.84%) were resistant to chloramphenicol.[15] Oyagade and Oguntoyinbo[16] found a 2%, 22%, 32% and 58% sensitivity to penicillin, ampicillin, tetracycline and chloramphenicol respectively which were also relatively low. Iroegbu et al.[17] in their study showed a sensitivity of 43.3%, 58.6%. 41.4% and 30.9% to penicillin, ampicillin, tetracycline and chloramphenicol respectively by their *Staphylococcus aureus* strains.

Florfenicol has been licensed exclusively for use in veterinary medicine. In the European Union, it has been approved for the treatment of respiratory tract infections in cattle in 1995 and in swine in 2000. However, in several non-European Union countries florfenicol is also licensed for the treatment of infectious pododermatitis in cattle and various bacterial diseases of commercially reared fish.[18]

A total of 23 chloramphenicol resistant MRSA isolates were tested for the presence of the *cfr* gene by PCR, 34.7 % of isolates were positive for this gene . Kehrenberg and Schwarz[14] demonstrated that at least one of genes (*fexA* and *cfr*)was present in all 11 staphylococcus isolates of animal origin . For isolates of human clinical origin, the first documented strain to harbour a chromosomally located *cfr* gene was MRSA strain CM05, isolated from a patient in Medellin, Colombia, in 2005.[19] Subsequent analysis showed that *cfr* also mediates resistance to other antimicrobial agents that bind to the same region of the ribosome, such as pleuromutilins, streptogramin A antibiotics, and—most importantly— oxazolidinones.[20] Studies of the distribution of *cfr* confirmed its presence mostly on plasmids which occasionally also carried *fexA* or other resistance genes, in various coagulase-negative staphylococci.[21,22] The *cfr* gene is currently the only gene that confers transferable resistance to oxazolidinones in clinical isolates, although cfr-like genes have recently been identified in environmental members of the order Bacillales and shown also to confer the PhLOPSA phenotype.[23]

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