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Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a Korean hospital

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Mupirocin has been used against Gram-positive pathogenic bacteria, and is a specific inhibitor of bacterial isoleucyl-tRNA synthetase. In this work, we have determined the prevalence of mupirocin resistance among staphylococci isolated from a Korean hospital, and have investigated the characteristics of the resistance. In *Staphylococcus aureus*, the prevalence of high-level mupirocin resistance was 5% (16 of 319), whereas low-level mupirocin resistance was not detected. In coagulase-negative staphylococci (CoNS) the rates of high- and low-level mupirocin resistance were 16.7% (34 of 204) and 10.3% (21 of 204), respectively. The high-level resistant strains contained the *ileS-2* gene, which encodes a novel staphylococcal isoleucyl-tRNA synthetase. In contrast, all of the low-level mupirocin-resistant CoNS contained the mutation V588F, which is located near the conserved motif KMSKS, within the chromosomal staphylococcal isoleucyl-tRNA synthetase gene (*ileS*). In conclusion, this work describes the recent, but rapid, emergence of two different types of mupirocin-resistant staphylococci in Korea, and the sequence and mutant characterization of the isoleucyl-tRNA synthetase of CoNS.

Keywords: mupirocin, staphylococci, resistance mechanism

Introduction

Mupirocin (pseudomonic acid A) specifically binds to bacterial isoleucyl-tRNA synthetase (IRS) and inhibits protein synthesis. Although the reaction intermediate of IRS, isoleucyl-adenylate and mupirocin have significantly different chemical structures, their interactions with the enzyme involve many common amino acids at the catalytic site.[1] As mupirocin is preferentially active against Gram-positive pathogens, it is clinically used as a topical antibacterial agent against staphylococci, including methicillin-resistant *Staphylococcus aureus* (MRSA) and streptococci.[2] Mupirocin became available in 1985, and subsequently has been used widely for the management of infection.[3] The increased use of this antibiotic has been accompanied by outbreaks of MRSA resistant to mupirocin, although the frequency of resistance is still low.[3] Nonetheless, the discovery of mupirocin-resistant strains indicates that a burst of resistant strains may soon emerge.

Mupirocin-resistant strains are divided into two groups: low- and high-level resistance (MICs 8–256 and >256 mg/L, respectively).[4] Low- and high-level resistance have been detected in both *S. aureus* and coagulase-negative staphylococci (CoNS).[4] In most cases, low-level resistance to mupirocin is related to alterations in the host IRS.[5,6] The clinical isolates resistant to a high level of mupirocin contain two distinct IRS enzymes: endogenous IRS plus an additional IRS encoded by the *ileS-2* gene.[7] This additional enzyme is usually encoded by transferable plasmids.[8–11] The IRS encoded by the *ileS-2* gene shares only 30% amino acid sequence similarity with the endogenous IRS of *S. aureus*.[12]

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A survey of mupirocin susceptibility of Gram-positive pathogens isolated in Korea up to 1999 failed to detect mupirocin-resistant staphylococci.[13] However, we considered it necessary to continue to monitor the emergence of the resistant strains, because the resistance rates of the reference antibiotics, such as methicillin and erythromycin, against Gram-positive bacteria are much higher than those of other countries,[14] and mupirocin ointment has been used widely for the management of skin infections in Korea.

To determine the prevalence of mupirocin resistance in a Korean hospital, we investigated the rates of mupirocin resistance among the clinical isolates of staphylococci. The characteristics of the resistant strains were then analysed in terms of their resistance level and genotype.

Materials and methods

Collection of clinical isolates

A total of 523 clinical isolates of Gram-positive cocci, comprising 319 *S. aureus* and 204 CoNS, were collected from the Severance General Hospital, in Seoul, Korea, between January 2000 and January 2002. The strains were associated with bacteraemia, hospital-acquired pneumonia, or skin and soft tissue infections. Only one isolate per patient was used, in order to avoid strain duplication. In the antibiogram typing analysis according to NCCLS standards,[15] these isolates showed different sensitivities to the tested drugs, including ciprofloxacin, kanamycin, tetracycline, vancomycin, erythromycin and methicillin, indicating that they are independent variants. The strains were stored in brain–heart infusion broth plus 20% glycerol at –70°C until studied. Mupirocin was obtained from Hanmi Pharmaceutical Co., Ltd, Korea.

Determination of MICs

MICs were determined by a standardized agar dilution method with Mueller–Hinton agar.[15] A microinoculator (Sakuma Co. Ltd, Tokyo, Japan) was used to inoculate the bacterial suspensions (10^4 cfu/spot). The stock solutions of test compounds were diluted in sterilized distilled water to give a serial, two-fold series, yielding final drug concentrations that ranged from 0.016 to 1024 mg/L. MIC determination was evaluated according to NCCLS standards.[15] *S. aureus* ATCC 29213 was used as the control for the susceptibility test.

PCR amplification and sequence analysis of the ileS-2 gene

A G-spin genomic DNA extraction kit (INtRON Biotechnology, Korea) was used to isolate genomic DNA. To detect the *ileS-2* gene, a 456 bp region in the *ileS-2* gene was amplified by PCR, using the primers 5′-TATATTATG-CGATGGAAGGTTGG-3′ and 5′-AATAAAATCAGCTG-GAAAGTGTTG-3′.[16] PCR was performed with 30 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 60 s and extension at 72°C for 60 s, by using 2U of *Vent* DNA polymerase (New England Biolabs, Beverly, MA, USA). The reaction products were analysed using 1.2% agarose gel electrophoresis. To confirm its identity, an entire sequence analysis of the *ileS-2* gene was performed, in two randomly selected isolates of *S. aureus* highly resistant to mupirocin. The entire *ileS-2* gene was amplified using two oligonucleotide primer pairs; one pair was Mup1 5′-CCCATGGCTTACCAGT-TGA-3′ and Mup2 5′-CCATGGAGCACTATCCGAA-3′,[17] and the other Mup3 5′-TTCGGATAG TGCTCCATG-3′ and Mup4 5′-CCCCAGTTACACCGATAT-3′.[18]

Sequence analysis of the host ileS gene

Genomic DNA, isolated to detect the *ileS-2* gene, was also used in this analysis. The primer pair SA *ileS*13D (5′-GA-TTTCCCAATGCGAGGTGGTTTACCAAACAAGGAAC CGC-3′) and SA *ileS*2833V (5′-CAACTTGTTGGCATCG-TGGGATAGATGCGTCAATTCATC-3′) was designed to amplify the entire coding sequence of the *S. aureus ileS* gene encoding the host IRS (GenBank accession no. X74219). The primer pair SE *ileS*10P (5′-GCCGAAAACTGATTTTCCT-ATGAGAGGTGGCTTACC-3′) and SE *ileS*2436R (5′-CG-TGCTTGTTCTAATGCACGGTTAACATCATCACG-3′) was designed for the entire host IRS coding sequence of CoNS. (The sequence was submitted to GenBank, accession no. AF516209.) PCR was performed using 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 60 s and extension at 72°C for 90 s, by using 2U of *Vent* DNA polymerase.

Results and discussion

Emergence and distribution of mupirocin resistance in staphylococci

The results are summarized in Table 1. Of the 319 clinical isolates of *S. aureus*, 237 (74.3%) were methicillin resistant, and of the 204 CoNS isolates, 163 (79.9%) were methicillin resistant. In *S. aureus*, high-level mupirocin resistance was detected in 16 (5%) of the isolates, of which 15 were methicillin resistant. Low-level mupirocin resistance was not detected.

Among the CoNS isolates, low-level mupirocin resistance was detected in 34 (16.7%), of which 30 were methicillin resistant. High-level mupirocin resistance was detected in 21 (10.3%) isolates, of which 17 were methicillin resistant. The mupirocin-resistant strains were not detected until 1999 in Korea, so these mutants seem to have emerged recently. In 1998, Schmitz *et al.*[3] studied staphylococci from 19 European hospitals, and found that the prevalence of high-level mupirocin resistance was 1.6% in *S. aureus* and 5.6% in CoNS.

Table 1. The distribution of low-level (LL) and high-level (HL) mupirocin resistance in clinical isolates of *S. aureus* and coagulase-negative staphylococci

Organism	No. (%) of isolates			
	total	susceptible	LL resistance	HL resistance
S. aureus	319 (100)	303 (95.0)	0	16 (5.0)
MRSA	237 (74.3)	222 (69.6)	0	15 (4.7)
MSSA	82 (25.7)	81 (25.4)	0	1 (0.3)
CoNS	204 (100)	149 (73.0)	34 (16.7)	21 (10.3)
MRCoNS	163 (79.9)	116 (56.9)	30 (14.7)	17 (8.3)
MSCoNS	41 (20.1)	33 (16.1)	4 (2.0)	4 (2.0)

LL resistance, low-level resistance (MIC 8–256 mg/L); HL resistance, high-level resistance (MIC > 256 mg/L).

Table 2. MIC of mupirocin, multiple drug resistance and genotyping of high-level mupirocin-resistant isolates of staphylococci

Isolates	Mupirocin MIC (mg/L)	*ileS-2* gene	Resistance type
S. aureus			
R196	4096	+	CIP, KAN, ERY, MET
P309	1024	+	KAN, ERY, MET
P443	1024	+	CIP, KAN, ERY, MET
P371	1024	+	KAN, MET
T130	1024	+	CIP, KAN, TET, ERY, MET
C292	1024	+	CIP, KAN, TET, ERY
C234	2048	+	CIP, KAN, ERY, MET
8035	1024	+	CIP, KAN, TET, ERY, MET
B1608	1024	+	CIP, KAN, ERY, MET
P28	1024	+	CIP, KAN, ERY, MET
CoNS			
P281	1024	+	KAN, TET, ERY, MET
C317	2048	+	KAN, MET
C1081	512	+	CIP, KAN, ERY, MET
C1089	1024	+	KAN, MET
C1090	512	+	CIP, KAN, ERY, MET
C1099	1024	+	CIP, TET, ERY, MET
C1103	1024	+	CIP, TET, ERY, MET
C1106	1024	+	CIP, KAN, TET, ERY, MET

CIP, ciprofloxacin; KAN, kanamycin; TET, tetracycline; ERY, erythromycin; MET, methicillin. The interpretive criteria were determined according to NCCLS.[15]

In a Greek hospital in 2001, five of 250 (2%) *S. aureus* isolates exhibited high-level resistance to mupirocin.[19] This study reveals that the mupirocin resistance rate in the Korean hospital is considerably higher than in other countries. It is also a serious concern that the resistance has reached this level in such a short time.

In the cases of both *S. aureus* and CoNS, the mupirocin resistance rates were higher in the methicillin-resistant than in the methicillin-susceptible isolates ($P < 0.05$). These results suggest that mupirocin resistance may be linked to an MRSA epidemic in the hospital. The connection between methicillin and mupirocin resistance has also been reported previously.[3,8]

ileS-2 gene in the high-level mupirocin-resistant strains

The *ileS-2* gene was detected in all of the high-level resistant isolates (Table 2). Thus, the acquisition of an additional gene, *ileS-2*, appears to be the major mechanism for the high-level

of mupirocin resistance in Korea. The *ileS-2* gene was not detected in any of the low-level resistant strains. The entire sequence analysis of the *ileS-2* gene, from the two isolates of the highly mupirocin-resistant *S. aureus*, showed that they are identical to those reported previously.[12]

Hodgson *et al.*[12] proposed that the *ileS-2* gene might have been imported from another organism, although database searches failed to identify a likely candidate. To infer the origin and the resistance mechanism of the *ileS-2* gene, we performed a sequence analysis with other IRS genes. In the alignment, the *ileS-2* sequence shared sequence identities of 44.5%, 30.8% and 29.5% with *Clostridium perfringens*, *S. aureus* and *Escherichia coli* IRS genes, respectively (data not shown).

Point mutations in the host IRSs of the low-level resistant strains

A comparison of endogenous *ileS* sequences from highly mupirocin-resistant isolates with the published sequence (*S. aureus ileS*, GenBank accession no. X74219) revealed no point mutation. This confirmed that endogenous IRS is not responsible for high-level mupirocin resistance. The molecular reason for low-level resistance in CoNS was revealed when we isolated the structural gene encoding the host IRS from *S. epidermidis* and determined its DNA sequence. (The sequence was submitted to GenBank, accession no. AF5-16209.) A comparison of the *ileS* sequences—segregated from low-level mupirocin-resistant isolates of CoNS—with the wild-type sequence revealed substitutions at several sites. Many of the substitutions were silent and encoded the same amino acids (data not shown). Interestingly, all of the low-level mupirocin-resistant CoNS isolates contained a point mutation encoding the change Val-588 to Phe (Table 3). Val-588 is located seven amino acids upstream of the region encoding the evolutionarily conserved KMSKS motif. The same mutation was also reported in the host IRSs of low-level mupirocin-resistant *S. aureus*.[5] The amino acid residues next to Val-588 in the staphylococcal IRSs are important for the interaction with mupirocin in other species.[1,20] In fact, these residues were mutated in mupirocin-resistant IRSs in *E. coli*[20] and *Methanosarcina barkeri*.[21] In the crystal structure of *S. aureus* IRS with mupirocin, the amino group of Val-588 makes a specific hydrogen bond with the oxygen in the carbonyl ester group of mupirocin.[22] Therefore, the amino acid change at Val-588 in CoNS is also believed to cause low-level resistance to mupirocin. In two strains, additional subsitutions were found at Arg-121 and Val-605 (Table 3); however, these residues are located far from the mupirocin binding region or active site, suggesting that they are not responsible for the resistance to mupirocin.

Table 3. Point mutations in the endogenous IRS gene of low-level mupirocin-resistant CoNS

	Mutations		
S. epidermidis	R121K	V588F	V605G
T29	–	+	–
C1093	–	+	+
C2019	+	+	–
C2023	–	+	–
T157	–	+	–
T204	–	+	–

In this work, we have determined the incidence of mupirocin resistance in a Korean hospital. The resistance rate is relatively high compared with that seen in other countries, and is expected to increase further, since this rate was reached in only 2 or 3 years. All of the high-level mupirocin-resistant strains have acquired the *ileS-2* gene, encoding a novel staphylococcal IRS that is most similar to those of *Clostridium* species. We also determined the host IRS sequence of *S. epidermidis* and investigated the mechanism for low-level mupirocin resistance. The sequence of the *S. epidermidis* IRS showed 83.0% identity with that of *S. aureus*. All of the low-level mupirocin-resistant strains of *S. epidermidis* possessed mutations at the endogenous IRS, which is identical to that found in low-level resistant strains of *S. aureus*. We are currently investigating the incidence of mupirocin resistance, including other hospitals serving different areas of Korea, to understand better the mupirocin resistance epidemic and the status of the unnecessary abuse of mupirocin.

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