ORIGINAL ARTICLE

In-Vitro Activity of Quinupristin/ Dalfopristin, Levofloxacin and Moxifloxacin Against Fusidic Acid and Rifampicin-Resistant Strains of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) from Malaysian Hospitals

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Summary

The in-vitro susceptibility of quinupristin/dalfopristin, levofloxacin and moxifloxacin against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which are also resistant to fusidic acid and rifampicin were carried out to determine whether these antibiotics can be used as an alternative treatment for multiply resistant MRSA strains. The minimum inhibitory concentrations (MIC) of these antibiotics were determined by E-test. Quinupristin/dalfopristin had good activity (MIC_{90} = 1mg/L) against these strains while most of the strains showed intermediate resistance to moxifloxacin with MIC_{90} = 2mg/L). However, more than 90% of these strains were resistant to levofloxacin with the MICs that ranged from 8 mg/L to 16 mg/L with the majority inhibited at 8 mg/L.

Key Words: Quinupristin/dalfopristin, Levofloxacin, Moxifloxacin • MIC • MRSA

Introduction

In Malaysia, the treatment of choice for serious MRSA infection is vancomycin. However, a combination of fusidic acid and rifampicin is used as an alternative oral antibiotic regimen for the treatment of bacteraemia, musculoskeletal and cardiovascular infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA)[1]. Fusidic acid and rifampicin are used in combination to prevent the emergence of resistance which may occur if these antibiotics are used individually. The resistance rates for fusidic acid and rifampicin individually were reported to be within the range of 3-5% in the years 1992 to 1996[2]. Resistance to both antibiotics will also limit the therapeutic options available as these antibiotics provide an oral alternative or follow-up therapy to vancomycin in Malaysia.

The novel semisynthetic injectable streptogramin quinupristin/dalfopristin offers a promise of effective treatment against MRSA. Quinupristin/dalfopristin are unrelated molecules that act synergistically against bacteria and therefore the possibility of selection of variants resistant to both components is reduced[3]. Quinupristin/dalfopristin are active against both methicillin-sensitive and MRSA with the MIC_{90} values for both bacteria being 1.0 mg/L[4]. The resistance of

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Staphylococcus aureus to other antibiotics did not affect the in-vitro activity of quinupristin/dalfopristin[5].

Moxifloxacin and levofloxacin are two new quinolones available in the market. Prado et al.[6] conducted a study which showed that methicillin susceptible *Staphylococcus aureus* (MSSA) were susceptible to both moxifloxacin and levofloxacin while MRSA was resistant to all. Similar findings was also noted by Fujiue et al.,[7] in which Gram-positive bacteria, MRSA and *Enterococcus faecalis* showed low susceptibility to levofloxacin, while methicillin susceptible *Staphylococcus aureus* (MSSA) and *Streptococcus pneumoniae* were highly sensitive to this drug.

This study was carried out to determine the in-vitro susceptibility of these antibiotics against our local MRSA strains that are resistant to both fusidic acid and rifampicin and to determine whether these antibiotics can be used as an alternative oral treatment for multiply resistant MRSA in Malaysia.

Materials and Methods

A total of 685 single patient's MRSA strains were obtained from 9 major hospitals situated in different geographical areas in Malaysia and collected from 1997 until 1999. These strains were tested against fusidic acid and rifampicin by disc diffusion testing following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines[8]. Out of these, 32 strains were shown to be resistant to both antibiotics. These strains were isolated from skin and wound swabs (47%), pus (28%), blood (9%), tracheal aspirates (6%), sputum (3%), urine (3%) and tissue (3%).

The resistance was confirmed by MIC determination using agar dilution method. Serial two-fold dilutions of fusidic acid were added to Mueller Hinton II agar to make up concentrations ranging from 256 mg/L to 1 mg/L. The same procedure was carried out for the preparation of rifampicin antibiotic plates. Strains were considered resistant to fusidic acid if the MIC was ≥ 4 mg/L as described by Toma and Barriault[9] and resistant to rifampicin if MIC was ≥ 4 mg/L as described in NCCLS guidelines. All the 32 strains were resistant to both antibiotics with the MIC of ≥ 4 mg/L.

The MIC for quinupristin/dalfopristin, levofloxacin and moxifloxacin was carried out using Etest strips (AB Biodisk, Sweden) following the guidelines outlined by the manufacturer. The MIC of each antibiotic was read where the zone of inhibition intersected with the strip. For quinupristin/dalfopristin the MICs of ≤ 2 mg/L represent susceptibility and MICs of ≥ 4 mg/L indicate resistance[10]. For levofloxacin, MIC of ≤ 2 mg/L is interpreted as sensitive and MIC ≥ 8 mg/L is considered resistant[11]. For moxifloxacin, U.S. Food and Drug Administration (FDA) breakpoints were used where MIC ≤ 1 mg/L is susceptible, 2 mg/L is intermediate and ≥ 4 mg/L is resistant[12].

Results

Of the 32 MRSA strains that were resistant to both fusidic acid and rifampicin, all were found to be sensitive to quinupristin/dalfopristin, with the MICs that ranged from 0.25 mg/L to 1 mg/L. More than 59% of the strains were inhibited at MIC 0.5 mg/L, while 38% were inhibited at MIC 1mg/L. The MIC_{90} of quinupristin/dalfopristin against these strains were 1 mg/L.

For levofloxacin susceptibility testing, these strains had MICs that ranged from 0.25 to 16 mg/L. Only 6% of these strains were susceptible while resistance was observed in 94% of the strains. Seventy-two percent of the resistant strains were mainly inhibited at 8 mg/L (72%) while 22% were inhibited at 16 mg/L. The MIC_{90} of levofloxacin against these strains were 16 mg/L.

The MICs of moxifloxacin for the fusidic acid and rifampicin resistant strains ranged from ≤ 0.06 mg/L to 4.0 mg/L. Percentage of strains susceptible to moxafloxacin was observed in 28% of the strains with the range of ≤ 0.06 mg/L to 1.0 mg/L. Most of the strains (69%) showed intermediate susceptibility (MIC 2mg/L). The MIC_{90} of moxifloxacin against these strains were 2 mg/L. The MIC distributions for quinupristin/dalfopristin, levofloxacin and moxifloxacin for fusidic acid and rifampicin resistant strains is shown in Table I.

Table I: MIC distribution of quinupristin/dalfopristin, levofloxacin and moxifloxacin for fusidic acid and rifampicin-resistant MRSA strains

	Number of strains inhibited [MIC (mg/L)]									
Antibiotic	≤0.06	0.125	0.25	0.5	1	2	4	8	≥16	% R
QD	0	0	1	19	12	0	0	0	0	0
Levo	0	0	1	0	1	0	0	23	7	94
Moxi	1	1	0	0	7	22	1	0	0	3

QD, Quinupristin/dalfopristin; Levo, Levofloxacin; Moxi, Moxifloxacin

Discussion

The emergence of multiantibiotic resistant MRSA and vancomycin-intermediate resistant *Staphylococcus aureus* has given new urgency to the development of new antimicrobial agents. Quinupristin/dalfopristin is active against most gram-positive pathogens[13,14]. Several studies had reported its efficacy in the treatment of MRSA infections[15,16]. In experimental endocarditis, it is found to act synergically with beta-lactams and is able to prevent the growth of highly beta-lactam-resistant MRSA in vivo[17]. Drew et al.[18] studied the safety and efficacy of quinupristin-dalfopristin in the treatment of a variety of infections due to MRSA in patients either intolerant of or failing prior therapy and suggested it as a treatment option for infections caused by MRSA, especially in patients intolerant of or failing alternate therapy. In a study from Taiwan, all MSSA isolates were susceptible to quinupristin-dalfopristin but high rates of nonsusceptibility (31%) to quinupristin-dalfopristin (MICs ≥ 2μg/ml) were demonstrated for MRSA. The use of virginiamycin in animal husbandry for more than 20 years in Taiwan could have contributed to the quinupristin-dalfopristin resistance[19]. Even though we did not test more isolates of MRSA against quinupristin/dalfopristin, all the fusidic acid and rifampicin resistant MRSA strains tested, which were also resistant to gentamicin, erythromycin, and cotrimoxazole, were susceptible to quinupristin/dalfopristin at the MIC_{90} of 1 mg/L. In this study, quinupristin/dalfopristin is the most active antibiotic when compared to moxifloxacin and levofloxacin against fusidic acid and rifampicin-resistant MRSA strains.

Several studies had demonstrated the good activity of levofloxacin against MRSA. In a study by Siegrist et al,[20] a high percentage of *Staphylococcus aureus* and MRSA was found to be susceptible to levofloxacin as most of other common clinical isolates. They suggested that it could be a promising therapeutic alternative for the treatment of Gram-positive infections. In an animal endocarditis therapeutic experiment, levofloxacin was found to be superior to ciprofloxacin and was at least equivalent to that of the standard treatment for MSSA or MRSA with either flucloxacillin or vancomycin[21]. This study, showed that levofloxacin exhibited the least activity compared to quinupristin/dalfopristin and moxifloxacin. More than 90% of the fusidic acid and rifampicin resistant strains were also resistant to levofloxacin with the MICs that ranged from 8 mg/L to 16 mg/L with the majority inhibited at 8 mg/L. This is in agreement with the studies conducted by Prado et al and Fujiue et al[6,7] which showed that this antibiotic has lesser efficacy against methicillin-resistant *Staphylococcus aureus.*

Malathum et al.[22] conducted a study on the in vitro activity of moxifloxacin against 189 gram-positive bacteria including *Staphylococcus aureus*, which showed the greater activity of moxifloxacin compared to ciprofloxacin with the minimal inhibitory concentrations (MICs) lower than those of ciprofloxacin by 2- to 64-fold. This improved activity was most prominent for *Staphylococcus aureus.* In this study, the majority of these MRSA strains showed intermediate susceptibility to moxifloxacin (MIC 2.0 mg/L). Resistance to moxifloxacin is low if compared to levofloxacin. This result suggested that a higher dose of moxifloxacin should be given to MRSA infected patients if they are to be treated with this antibiotic. However, we stress that despite the in-vitro activity exhibited, quinolones should not be a drug of first choice for the treatment of MRSA infection in view of the emergence of resistance. If there is a need to use a quinolone it should be used in combination with another antistaphylococcal agent.

In conclusion, quinupristin/dalfopristin showed good in-vitro activity against MRSA strains that are resistant to both fusidic acid and rifampicin. The majority of MRSA in this study exhibited intermediate susceptibility to

moxifloxacin. This could have some implications when determining the optimum dosage in the use of moxifloxacin in the treatment of MRSA infections in Malaysia. Levofloxacin showed the least susceptibility in these strains and is therefore not the alternative treatment of choice.

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