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Comparison of *in vitro* susceptibilities to levofloxacin and ciprofloxacin with *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolated from cystic fibrosis patients in Northern Ireland

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In vitro antibiotic susceptibility (E-test) studies were performed on *Pseudomonas aeruginosa* (n=50) and *Stenotrophomonas maltophilia* (n=10) obtained from the sputa of adult and paediatric patients with cystic fibrosis (CF). In general, *S. maltophilia* showed greater susceptibility to levofloxacin than to ciprofloxacin, which was in contrast to the susceptibility of *P. aeruginosa*, which showed that generally isolates were more susceptibility to ciprofloxacin than to levofloxacin. As the optimal treatment of CF-related infections due to these organisms is not fully known, these data may help in deciding upon appropriate empirical oral fluoroquinolone treatment of *P. aeruginosa* and *S. maltophilia* in CF patients.

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Cystic fibrosis is the most common fatal genetic disease of Caucasians and has an approximate incidence of 1 in 2500 live births and a carriage rate of 1 in 20 individuals. Patients with CF suffer recurrent and chronic respiratory tract infections and most of the associated morbidity and mortality is due to such infections throughout their life.[1] These chronic infections are usually dominated by multidrug-resistant Gram-negative organisms, especially the pseudomonads, particularly *P. aeruginosa*; however. other multidrug-resistant organisms, including *S. maltophilia*, have recently been reported to be more prevalent.[2] A comprehensive review of these organisms may be found elsewhere.[3,4]

Growing antibiotic resistance in *P. aeruginosa* and *S. maltophilia* isolated from the sputa of adult and paediatric patients with CF is a major problem in CF centres in Northern Ireland, across Britain and worldwide. Furthermore, as there is relatively little information in the literature on the comparison of the *in vitro* susceptibility of Gram-negative organisms to the fluoroquinolones, any study examining potential improvements in efficacy should be encouraged.

P. aeruginosa isolates (n=50) from 45 patients (15 adults [8 female, 7 male]; 30 paediatric patients [16 male, 14 female]) with well-characterised CF were obtained from freshly expectorated sputum specimens. In addition, *S. maltophilia* (n=10) isolates from an equal number of adult patients with CF (5 females, 5 males]) were obtained.

The identity of these isolates was confirmed phenotypically by the API20NE scheme (bioMerieux, Les Halles, France) and genotypically in the case of *P. aeruginosa* isolates by polymerase chain reaction (PCR) amplification of the *groES* gene locus, as previously described.[5] An additional phenotypic confirmation assay was performed on all *S. maltophilia* isolates by examining for synergy between augmentin and azotreonam discs.

The minimum inhibitory concentrations (MICs) of levofloxacin and ciprofloxacin were determined by the E-test method (AbBiodisk), following the manufacturer's instructions, and all assays were performed on Mueller-Hinton agar (Oxoid, Dorset, UK). Susceptibility breakpoints were taken as ≤ 2 μg/mL for levofloxacin and ≤ 1 μg/mL for ciprofloxacin, according to NCCLS guidelines.[6] Antibiotic susceptibility of the 50 *P. aeruginosa* isolates and 10 *S. maltophilia* isolates, to the two fluoroquinolones tested, are shown in Table 1.

The E-test method was selected because of its reliability in testing susceptibility to the fluoroquinolones,[7] its ease of use and its definition of the MIC.[8] In general, *S. maltophilia* showed greater susceptibility to levofloxacin than to ciprofloxacin, as demonstrated by: a) fewer *in vitro* resistant isolates recorded for levofloxacin versus ciprofloxacin; b) a diminished range of MICs recorded for levofloxacin compared to ciprofloxacin; and c) reduced MIC_{50} and MIC_{90} values recorded for levofloxacin compared to ciprofloxacin.

This contrasts to the susceptibility of *P. aeruginosa*, where isolates generally showed a greater susceptibility to ciprofloxacin than to levofloxacin. Furthermore, there was no marked difference in susceptibility of *P. aeruginosa* isolated from either patient group studied.

Two isolates of *P. aeruginosa* and one isolate of *S. maltophilia*

Table 1. Comparison of susceptibility to levofloxacin and ciprofloxacin of 50 *P. aeruginosa* isolates and 10 *S. maltophilia* isolates obtained from patients with cystic fibrosis.

Species	Number of isolates examined	Levofloxacin				Ciprofloxacin			
		Number (%) of resistant isolates	Range of MICs	MIC_{50}	MIC_{90}	Number (%) of resistant isolates	Range of MICs	MIC_{50}	MIC_{90}
S. maltophilia	10	1 (10%)	0.19-6.0	0.26	2.21	3 (30%)	0.25->32	0.67	12.02
P. aeruginosa (adults)	20	7 (35%)	0.047->32	1.35	25.0	6 (30%)	0.023->32	0.39	17.31
P. aeruginosa (paediatric)	30	11 (55%)	0.094->32	1.50	12.0	13 (65%)	0.125-12	0.75	4.38

Units for all MIC_{50} and MIC_{90} values are μg/mL.

demonstrated high-level resistance (>32 μg/mL) to ciprofloxacin, while four isolates of *P. aeruginosa* demonstrated high-level resistance (>32 μg/mL) to levofloxacin. In all cases, the MIC_{50} values for both levofloxacin and ciprofloxacin were below the breakpoints for both *S. maltophilia* and *P. aeruginosa*, whereas MIC_{90} values for both organisms were above their respective breakpoints. Additionally, decreased susceptibility was noted more often for *P. aeruginosa* with levofloxacin than with ciprofloxacin. This demonstrates the potential for cross-resistance among the fluoroquinolones, as levofloxacin has not been employed to date in the Northern Ireland CF patient population, whereas ciprofloxacin has been used widely.

It is interesting to note from this study that although *S. maltophilia* generally is regarded as a more intrinsically resistant organism than *P. aeruginosa*, mainly due the presence of a carbapenemase and a penicillinase, *S. maltophilia* exhibited lower susceptibility to the fluoroquinolones than did *P. aeruginosa*.

Taxonomically, *S. maltophilia* was classified originally as *P. maltophilia*, but was reclassified in 1983 as *Xanthomonas maltophilia*[9] and then again in 1993 as *Stenotrophomonas maltophilia*.[10] Although it shares phylogeny with *P. aeruginosa* (approximately 80% similarity of the 16S rRNA gene), the basis for the lowered susceptibility of *S. maltophilia* to the fluoroquinolones differs from that of *P. aeruginosa*, in which fluoroquinolone resistance in *S. maltophilia* is believed to be due to overproduction of efflux mechanisms and not due to mutations in the quinolone-resistance determining region (QRDR). Recently, Ribera *et al*.[11] were unable to demonstrate mutations in the *gyrA* and *parC* gene loci of fluoroquinolone-resistant *S. maltophilia* isolates.

This is in contrast to *P. aeruginosa*, in which the major mechanism of resistance to fluoroquinolones is modification of the type II topoisomerases DNA gyrase and topoisomerase IV.[12] Although previous studies have demonstrated mutations in the QRDR of the *gyrA*, *gyrB*, *parC*, and *parE* genes, most notably as substitutions of Ile for Thr-83 in *gyrA* and Leu for Ser-87 in *parC*, the sequence of and frequency with which these QRDR mutations occur is not well elucidated in fluoroquinolone-resistant *P. aeruginosa*,[12] unlike the case with fluoroquinolone-resistant pneumococci.[13]

Further studies are needed to uncover the mechanisms by which QRDR mutations are switched on in *P. aeruginosa* in relation to specific fluoroquinolone, in order that those fluoroquinolones that switch on such QRDR mutations (and hence resistance) rapidly are avoided in favour of fluoroquinolones with slow or only limited QRDR mutation potential.

Recent data from the US CF Synergy Testing Laboratory, based at Columbia University, New York, showed that ciprofloxacin used in combination with ticarcillin–clavulanate had a synergistic effect whereby 64% of isolates were inhibited, while a combination of ciprofloxacin and piperacillin–tazobactam inhibited 59% of isolates. This suggests that fluoroquinolones may have a role not only in monotherapy but also when used in synergistic combination with other classes of antimicrobial agents.[2]

In summary, as the optimal treatment of CF-related infections due to the two organisms studied here is not fully known, these data may help when deciding on appropriate empirical treatment of *P. aeruginosa* and *S. maltophilia* infections with oral fluoroquinolones in CF patients. □

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Acquired immune deficiency syndrome and human immunodeficiency virus: Taiwanese medical laboratory technologists and students' attitudes, concerns and knowledge

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The World Health Organization (WHO) estimated that in 2003 that almost 38 million people worldwide were living with human immunodeficiency virus (HIV) infection.[1] Medical laboratory workers are the second largest healthcare science professional group with potential exposure to HIV through the handling of biological specimens.

Previous studies from the USA, New Zealand, South Pacific and Finland have shown consistently that medical laboratory workers have many unfounded fears about HIV and acquired immune deficiency syndrome (AIDS) that impact on their work practices and in their attitudes towards AIDS patients.[2–6] However, it would appear that no such studies have been undertaken in Asian countries where, according to WHO, prevalence of HIV is increasing more rapidly than in the Western world.

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Here, the attitudes and concerns and their relationship to AIDS/HIV knowledge of medical laboratory staff and students from central Taiwan are reported.

A questionnaire was circulated to all medical laboratory staff and medical laboratory technology students from four hospitals (Pingdon Christian Hospital, Show Chwan Memorial Hospital, Changhwa Christian Hospital and Changwa Show Chwan Hospital) and three medical laboratory technology schools (Chunghwa Medical Technology College, Chungtai Medical Technology College and Chung Shan Medical University) in central Taiwan. Questionnaires were distributed in January 2004 and collected in March 2004. The purpose of the study was explained to the laboratory staff and students and ethical permission was obtained from the appropriate ethical committees.

The questionnaire had been used previously in studies of laboratory staff and nurses,[3–5,7–9] and was based on a statistically validated questionnaire used to determine AIDS knowledge and attitudes in UK nurses.[10] The questionnaire consisted of four sections. In section one, demographic data, such as age, gender, gloving practices and previous attendance at AIDS/HIV lectures, was requested. In section two, the person's responses to a variety of concerns about handling biological specimens was requested. Section three presented four statements with five possible responses, ranging from 'strongly agree' to 'strongly disagree'. Section four tested the person's knowledge of methods for destroying HIV and in which biological specimens HIV could be detected.

The questionnaire was translated into Chinese and was answered by self-administration. Participation was voluntary and no personal information was collected, nor were the researchers aware of a respondent's identity. Data from the completed questionnaires were entered on to a database and analysed by Pearson's $\chi 2$ test or by Fisher's exact test where appropriate, with statistical significance set at $P=0.05$.

Of a potential pool of 530 persons, 380 (144 students and 236 medical laboratory technologists) returned questionnaires (71.7% response rate). There was no significant difference in response rates between students and medical laboratory technologists. Respondents were predominantly female (77.1%) and 196 (51.6%) had previously attended workshops or lectures on AIDS/HIV. Over 90% always wore gloves when handling a variety of biological specimens and 62.6% treated all specimens as potentially positive for HIV. There was a significant association between use of gloves and concern about acquiring HIV/AIDS or hepatitis at work ($P<0.001$).

Respondents showed concern about acquiring HIV or hepatitis B in their work place (84.2% and 77.1%, respectively) and 200 responded in the affirmative as to whether or not anyone in their immediate family expressed concerns about their work in relation to AIDS/HIV. Forty-four (18.6%) medical laboratory technologists were seriously considering leaving the profession because of possible contact with AIDS or HIV-positive patients or biological samples, while 67 (28.4%) expressed uncertainty about the matter. Of the 44 seriously considering leaving the