In vitro activity of quinolones against *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* in Saudi Arabia

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نشاط الكينولونات في المختبر ضد العقدية الرئوية، والمستدمية النزلية، والموراكسيلة النزلية، في المملكة العربية السعودية

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الخلاصة: تم في هذه الدراسة تحديد حساسية 88 من مستفرَدات جراثيم العقدية الرئوية *S. pneumoniae*، و116 من مستفرَدات المستدمية النزلية *H. influenzae*، و80 من مستفرَدات المواركسيلة النزلية *Moraxella catarrhalis*، لستة من الفلوروكينولونات، وهي: السيبرفلوكساسين، والأوفلوكساسين، والليفوفلوكساسين، والتروفافلوكساسين، والغريبافلوكساسين، والجيميفلوكساسين. وقد أُخذت هذه المستفرَدات من مرضى مصابين بأمراض مُغيرة في أربعة من مستشفيات المملكة العربية السعودية في المدة من عام 1996 إلى عام 1998. وبينت الدراسة أن مستفرَدات العقدية الرئوية لديها حساسية كاملة للتروفالوكساسين والغريبافلوكساسين والجيميفلوكساسين؛ وكانت حساسيتها للأوفلوكساسين 97.7٪، وحساسيتها لليفوفلوكساسين 98.9٪. أما مستفرَدات المستدمية النزلية فكانت حساسة لجميع العوامل، باستثناء التروفافلوكساسين (99.1٪). وأما ذراري المواركسيلة النزلية فكانت لديها حساسية كاملة لجميع العوامل، باستثناء الأوفلوكساسين (97.5٪). و لم تُكْشَف أية مستفرَدات مقاومة للجيميفلوكساسين أو الغريبافلوكساسين. وتوصي الدراسة بمواصلة الترصُّد لاكتشاف التغيرات في حساسية العوامل المسببة للمرض التي تؤدي إلى الالتهاب الرئوي المكتسب من المجتمع.

ABSTRACT Susceptibility of 88 clinical ***Streptococcus pneumoniae*** isolates, 116 ***Haemophilus influenzae*** isolates and 80 ***Moraxella catarrhalis*** isolates to 6 fluoroquinolones—ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, grepafloxacin and gemifloxacin—were determined. Isolates were from patients with invasive disease at 4 hospitals in Saudi Arabia between 1996 and 1998. ***S. pneumoniae*** isolates were fully susceptible to trovafloxacin, grepafloxacin and gemifloxacin; susceptibility to ofloxacin and levofloxacin was 97.7% and 98.9% respectively. ***H. influenzae*** isolates were susceptible to all agents, except for trovafloxacin (99.1%). ***M. catarrhalis*** strains were fully sensitive to all agents except ofloxacin (97.5%). No isolates were resistant to gemifloxacin or grepafloxacin.

Activité *in vitro* des quinolones vis-à-vis de *S. pneumoniae*, *H. influenzae* et *M. catarrhalis* en Arabie saoudite

RÉSUMÉ La sensibilité de 88 isolats cliniques de ***Streptococcus pneumoniae***, de 116 isolats d'***Haemophilus influenzae*** et de 80 isolats de ***Moraxella catarrhalis*** à six fluoroquinolones – ciprofloxacine, ofloxacine, lévofloxacine, trovafloxacine, grépafloxacine et gémifloxacine – a été déterminée. Les isolats provenaient de patients présentant une maladie invasive dans quatre hôpitaux d'Arabie saoudite entre 1996 et 1998. Les isolats de ***S. pneumoniae*** étaient très sensibles à la trovafloxacine, à la grépafloxacine et à la gémifloxacine ; la sensibilité à l'ofloxacine et à la lévofloxacine était de 97,7 % et 98,9 % respectivement. Les isolats de ***H. influenzae*** étaient sensibles à tous les agents sauf à la trovafloxacine (99,1 %). Les souches de ***M. catarrhalis*** étaient très sensibles à tous les agents sauf à l'ofloxacine (97,5 %). Aucun isolat n'était résistant à la gémifloxacine ou à la grépafloxacine.

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Introduction

Respiratory quinolones, as they are sometimes referred to, have become a class of their own among the quinolones because of their broad spectrum activity against Gram-positive, Gram-negative and atypical pathogens [*1*]. These newer fluoroquinolones like levofloxacin, grepafloxacin, trovafloxacin and gemifloxacin, even though they are not as effective as ciprofloxacin against *Pseudomonas spp.*, have improved activity against Gram-positive organisms, especially *Streptococcus pneumoniae*, thus making them more efficacious in the treatment of community-acquired pneumonia [*1–4*]. In Saudi Arabia, the only approved fluoroquinolones are ciprofloxacin, ofloxacin and norfloxacin. Only very recently did the Ministry of Health approve moxifloxacin for general prescription.

At present, there are only a few reports globally on resistance of common respiratory pathogens to the newer quinolones; none of these reports are from Saudi Arabia [*5,6*]. However, as there is no effective antibiotic control by the government in our country, it would not be surprising to find that these drugs may soon be overused. Even though the isolation and detection of fluoroquinolone-resistant *Haemophilus influenzae* strains have been rare, the respiratory quinolones need to be evaluated to determine their comparative clinical potencies and *in vitro* efficacy against *H. influenzae*, *Moraxella catarrhalis* and *S. pneumoniae* in each community.

The purpose of our study was to determine *in vitro* susceptibility patterns to ciprofloxacin and some of the newer quinolones among Saudi Arabian isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* and to ascertain if resistant strains have emerged. This will provide supportive data for the more effective use of all quinolone antibiotics, especially the new ones.

Methods

Between 1996 and 1998, 284 isolates of *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* were collected from patients with invasive diseases including pneumonia, bacteraemia and meningitis in the western, central and eastern provinces of Saudi Arabia. Patients were seen at King Fahad National Guard Hospital in Riyadh, King Saud University Hospital in Riyadh, King Khalid National Guard Hospital in Jeddah or Qatif Central Hospital in Qatif. No patients had taken antimicrobials for the 2 weeks preceding the study.

This was a multicentre survey in which non-repeat isolates were collected by each centre. Organisms were included if:

- They were obtained from fresh clinical material received in the laboratory.
- They were obtained from appropriate samples from patients with clinical indications of respiratory tract infections.
- They were isolated using routine methods.
- They were identified using conventional methods (Optochin susceptibility for *S. pneumoniae* and X and V factor requirement for *H. influenzae*. *M. catarrhalis* was identified using oxidase spot test and butyrate esterase disc).
- Strains were stored within 48 hours of isolation at –70 °C by inoculation into routine storage medium.

Organisms were excluded if they were isolated from throat swabs from healthy carriers or duplicate isolates from the same patient.

Isolates were inoculated into Mueller–Hinton (MH) broth, colonies were removed from 24-hour chocolate agar and the suspension was adjusted to 0.5 McFarland turbidity standard. A sterile cotton swab was dipped into the adjusted suspension and

used to inoculate the *Haemophilus* spp. test medium for *H. influenzae* strains. *S. pneumoniae* strains were plated on MH agar supplemented with 5% sheep cells and *M. catarrhalis* strains were plated on MH agar. Using a template, we applied antibiotic epsilometer (Etest) strips of the antibiotics to the agar surface (AB Biodisk, Solna, Sweden). After 18–24 hours incubation at 35 °C in 5% CO_2 for *H. influenzae* strains and in air for *S. pneumoniae* and *M. catarrhalis*, the minimum inhibitory concentration (MIC) values were read. We interpreted the elliptical zones of inhibition as per manufacturer's instructions, i.e. reading the value on the Etest strip where the thin end of the ellipse, or teardrop, intersected the strip. National Committee of Clinical and Laboratory Standards (NCCLS) criteria for breakpoint values were used to determine the susceptibility of isolates to the various antibiotics [*3,7*]. Susceptibility rates were determined as: ciprofloxacin ≤ 1.0 µg/mL, ofloxacin ≤ 2.0 µg/mL, levofloxacin ≤ 2.0 µg/mL, trovafloxacin ≤ 1.0 µg mL and grepafloxacin ≤ 0.5 µg/mL. For gemifloxacin the breakpoint was ≤ 0.25 µg/mL, which corresponded to a zone diameter of 20–39 mm [*3*]. The MIC_{50} and MIC_{90} were based on the cumulative MIC values of each organism against each antimicrobial agent. Tests of MIC values were controlled with organisms of known susceptibility. For *S. pneumoniae* testing, *Escherichia coli* ATCC 235218, *S. pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 were used with each batch on MH agar plus 5% sheep blood. For *H. influenzae* testing, *E. coli* ATCC 35218 and *H. influenzae* ATCC 49247 and ATCC 49766 were used with each batch on haemophilus test medium (HTM) agar. Results were collated from the combined data from Riyadh, Jeddah and Qatif.

Results

S. pneumoniae

Table 1 shows the MIC_{50} and MIC_{90} values for *S. pneumoniae* against each agent. Of the 6 agents, gemifloxacin exhibited the lowest MIC_{50} and MIC_{90} against *S. pneumoniae*, closely followed by grepafloxacin and trovafloxacin. The MIC_{90} of ofloxacin and ciprofloxacin were nearly the same at 2.0 mg/L and exceeded the breakpoint recommended by the NCCLS. The most active agent was gemifloxacin with an MIC_{90} of 0.06 mg/L.

Table 1 **Minimum inhibitory concentration (MIC) values for 88 isolates of *Streptococcus pneumoniae***

Antimicrobial	MIC (mg/L)			Susceptibility (%)	No. of isolates
	MIC_{50}	MIC_{90}	MIC range		
Gemifloxacin	0.03	0.06	0.008–0.25	100	88
Ciprofloxacin	1	2	0.03–4.0	98.9	87
Grepafloxacin	0.06	0.12	0.047–0.75	100	88
Levofloxacin	1	1	0.12–4.0	98.9	87
Ofloxacin	2	2	0.12–4.0	97.7	86
Trovafloxacin	0.06	0.12	0.015–0.5	100	88

Table 1 also shows the percentage of *S. pneumoniae* isolates susceptible to each antimicrobial. Among the strains of *S. pneumoniae*, the prevalence of fluoroquinolone resistance is low, but has been emerging to less potent agents such as ciprofloxacin, levofloxacin and ofloxacin. We identified 1 strain resistant to ciprofloxacin, 1 strain resistant to levofloxacin and 2 strains resistant to ofloxacin.

H. influenzae

Table 2 shows MIC_{50} and MIC_{90} values for test antimicrobials against *H. influenzae*. Each quinolone was active against *H. influenzae*. All 116 strains of *H. influenzae* demonstrated 100% susceptibility to all tested agents except trovafloxacin, which had a susceptibility rate of 99.1%. Gemifloxacin, ciprofloxacin, grepafloxacin and levofloxacin each had MIC_{90} of 0.03 mg/L against *H. influenzae*. Of these, gemifloxacin had the lowest MIC_{50} with a value of 0.008 mg/L. Trovafloxacin was the most active agent with MIC_{50} and MIC_{90} of 0.015 mg/L. Only 1 *H. influenzae* strain was resistant to trovafloxacin.

M. catarrhalis

Table 3 shows the MIC_{50} and MIC_{90} values of the quinolones for *M. catarrhalis*. Of all 6 agents, gemifloxacin and trovafloxacin had the lowest MIC_{90} values at 0.03 mg/L each, closely followed by ciprofloxacin, grepafloxacin and levofloxacin. Of the 80 *M. catarrhalis* strains tested, only 2 were resistant to ofloxacin. Therefore, of all agents tested, gemifloxacin had the lowest MIC values for all 3 pathogens and had the lowest overall MIC values against *S. pneumoniae*.

Discussion

The quinolones have become an important group of antibiotics over the past 2 decades since they were introduced into clinical use for the treatment of serious infections [*8,9*]. The older antimicrobials belonging to this group such as ciprofloxacin, ofloxacin and norfloxacin have potent *in vitro* activity against many Gram-negative organisms. However, their efficacy against some important Gram-positive organisms and atypical agents was below clinical expectations [*10*]. The new fluoroquinolones appear to have several advantages over their older prototypes, including a broader spectrum of activity as well as improved bioavailability and safety. In addition, they may offer shorter duration of therapy lead-

Table 2 **Mean inhibitory concentration (MIC) values for 116 isolates of *Haemophilus influenzae***

Antimicrobial	MIC (mg/L)			Susceptibility	No. of
	MIC_{50}	MIC_{90}	MIC range	(%)	isolates
Gemifloxacin	0.008	0.03	0.001–0.25	100	116
Ciprofloxacin	0.015	0.03	0.015–1.0	100	116
Grepafloxacin	0.015	0.03	<0.015–0.25	100	116
Levofloxacin	0.03	0.03	<0.015–1.0	100	116
Ofloxacin	0.06	0.06	<0.06–2.0	100	116
Trovafloxacin	0.015	0.015	<0.015–4.0	99.1	115

Table 3 **Mean inhibitory concentration (MIC) values for 80 isolates of *Moraxella catarrhalis***

Antimicrobial	MIC (mg/L)			Susceptibility (%)	No. of isolates
	MIC_{50}	MIC_{90}	MIC Range		
Gemifloxacin	0.015	0.03	0.004–0.12	100	80
Ciprofloxacin	0.03	0.06	<0.015–0.12	100	80
Grepafloxacin	0.015	0.06	0.015–0.25	100	80
Levofloxacin	0.06	0.06	0.03–0.25	100	80
Ofloxacin	0.012	0.25	<0.06–4.0	97.5	78
Trovafloxacin	0.015	0.03	<0.015–0.06	100	80

ing to improved compliance and cost savings [*1,11*]. In 1998, the Infectious Disease Society of America guidelines included the fluoroquinolones as an option in empirical management of community-acquired pneumonia due to their activity against intermediately and highly resistant strains of *S. pneumoniae* [*8,12*]. Concerns regarding the liberal use of these agents and the rapid emergence of resistance, especially among *S. pneumoniae*, however, have been raised [*13,14*].

Many reports and surveillances have documented the global emergence of resistance to penicillins, cephalosporins and macrolides among common agents causing community-acquired pneumonia [*15–17*]. The Alexander Project, which was begun in 1992 and included isolates from many countries including Saudi Arabia, found that the resistance of *H. influenzae* and *S. pneumoniae* isolates to ofloxacin and ciprofloxacin had not exceeded 1% and identified no *M. catarrhalis* quinolone-resistant strains [*18*]. Unfortunately more recent data are not as encouraging. The prevalence of *S. pneumoniae* with decreased susceptibility to fluoroquinolones is increasing [*19–21*]. When over 7000 *S. pneumoniae* isolates were examined between 1988 and 1998, the percentage of pneumococcal isolates with reduced susceptibility to fluoroquinolones increased from 0.5% in 1993–1994 to 2.0% in 1997–1998. In Canada, more resistant isolates were more common among people aged 65 years and older and among people from Ontario. This was associated with a 5-fold increase in the number of fluoroquinolones prescribed during 1993–1998 [*22*]. More reports have indicated the increasing resistance of *S. pneumoniae* isolates to the quinolones, including to the newer agents such as levofloxacin [*23,24*].

The resistance of *H. influenzae* to ampicillin appears to be steadily rising in Saudi Arabia from 4% in 1982 to 17% in 1994 [*25–28*]. The prevalence rate of *S. pneumoniae* resistance to penicillin and ampicillin has varied considerably by city, but in general it has dropped from 100% sensitivity in the early 1980s to below 40% in the last few years [*29–35*]. Sensitivity testing to penicillin has shown, however, that most resistant strains are intermediately resistant, while no more than 10% is highly resistant *S. pneumoniae* [*18,29,33*]. There is strong evidence that a number of strains resistant to agents commonly used to treat community-acquired pneumonia have emerged in our country and continue to become more common [*33*].

Since new quinolones provide a valid alternative antibacterial therapy, especially in areas where the prevalence of penicillin-resistant and macrolide-resistant organisms dominate, and since they have become licensed for the first time in the Kingdom of Saudi Arabia, we examined the local susceptibility pattern of the most common agents of community-acquired pneumonia to some of them. Among the 284 clinical isolates of *H. influenzae*, *S. pneumoniae* and *M. catarrhalis*, only 7 isolates were resistant (4 *S. pneumoniae*, 1 *H. influenzae* and 2 *M. catarrhalis*). This indicated excellent sensitivity among our local isolates and could be easily explained by the rare use of the newer quinolones since only ciprofloxacin is registered on the formulary of most hospitals in the country.

One *S. pneumoniae* strain was sensitive to levofloxacin but resistant to ciprofloxacin. Perhaps an active efflux mechanism in this particular strain resulted in lower resistance. In some strains of *S. pneumoniae*, an efflux protein, PmrA, mediates this mechanism [*36,37*]. In 2000, after the completion of our study, ciprofloxacin was widely used for the first time during the pilgrimage event as a decolonizing agent for returning pilgrims to protect their family against meningitis [*38*]. For this reason, it is expected that resistance to ciprofloxacin will gradually increase if the practice is continued or abused.

The literature on *M. catarrhalis* and its susceptibility to antibiotics in Saudi Arabia is scant. In Riyadh, 27 of 32 strains (84%) isolated from clinical specimens produced beta-lactamases and were resistant to erythromycin and clindamycin phosphate. All isolates, however, were susceptible to ciprofloxacin, tetracycline and trimethoprim–sulfamethoxazole [*39*]. In our study, the MIC_{90} was well below the breakpoint of the various quinolones tested (Table 3).

Although clinical studies are needed to confirm our *in vitro* results, resistance rates to each newer quinolone were too low to be of clinical concern. In a study from Canada, a high sensitivity rate to levofloxacin was documented, while other quinolones were not included [*40*]. Similarly, in the Alexander project, low rates of resistance to ciprofloxacin and cefuroxime were documented from 266 isolates collected between 1996 and 1997 [*18*].

Our findings suggest that gemifloxacin, grepafloxacin, trovafloxacin and levofloxacin would be effective agents in respiratory tract infections caused by the common pathogens in our country. Nevertheless, in order to maintain the efficacy of this group of antimicrobials, we should enforce their judicious use and implement continued surveillance of susceptibility of the different organisms to this class of fluoroquinolones.

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WHO Expert Committee on Specifications for Pharmaceutical Preparations, thirty-eighth report

This report presents the recommendations of an international group of experts convened by the World Health Organization to consider matters concerning the quality assurance of pharmaceuticals and specifications for drug substances and dosage forms. Of particular relevance to drug regulatory authorities and pharmaceutical manufacturers, this report discusses the latest volume of the *International pharmacopoeia* and quality specifications for pharmaceutical substances and dosage forms, as well as quality control of reference materials, good manufacturing practices, inspection, distribution and trade and other aspects of quality assurance of pharmaceuticals, and regulatory issues. The report is complemented by a number of annexes, including recommendations on good trade and distribution practices for pharmaceutical starting materials, guidelines on the WHO scheme for the certification of pharmaceutical materials moving in international commerce, draft procedures for assessing quality control laboratories and procurement agencies for use by the United Nations agencies, and guidelines for preparing a laboratory information file and a procurement agency information file. Further information on this publication can be obtained from WHO Press: http://www.who.int/bookorders/anglais/home1.jsp?sesslan=1