Journal of Antimicrobial Chemotherapy (1999) **43**, 607–612

JAC

Correspondence

Identification of *oprG*, a gene encoding a major outer membrane protein of *Pseudomonas aeruginosa*

J Antimicrob Chemother 1999; **43:** 607–608

Karl Gensberg[a], Anthony W. Smith[b], Fiona S. L. Brinkman[c] and Robert E. W. Hancock[c]*

[a]*Department of Surgery, Queen Elizabeth Hospital, Birmingham, UK;* [b]*Department of Pharmacy and Pharmacology, University of Bath, Bath, UK;* [c]*Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada*

*Corresponding author. Tel: +1-604-822-2682; Fax: +1-604-822-6041; E-mail: bob@cmdr.ubc.ca

Sir,

Pseudomonas aeruginosa expresses between five and nine major outer membrane proteins (OMPs), depending on the conditions of growth;[1,2] the genes encoding all but one of these, OprG, have been identified. OprG has an apparent molecular weight of 25,000 when outer membranes are heated to 88°C for 10 min in solubilization buffer before being subjected to SDS–PAGE and 19,000 when they are not pre-heated.[3] Its appearance in the outer membrane is highly dependent on growth conditions. In particular, Yates *et al.*[4] observed a direct relationship between the iron concentration in the medium and expression of OprG and suggested that this OMP is involved in low-affinity iron uptake. Other conditions, including growth into the stationary phase, higher growth temperatures, Mg^{2+} deficiency, certain lipopolysaccharide alterations and the presence of certain carbon sources, also result in the expression of varying concentrations of OprG.[1] Such apparently broad regulation of this OMP has frustrated efforts to assign a function to it. However, in addition to the potential role of OprG in iron uptake, two groups of investigators have suggested that it is involved in quinolone uptake/susceptibility.[4,5] In view of this uncertainty, we undertook to identify the *oprG* gene.

Outer membranes were prepared according to the method described by Hancock & Carey[3] from a 500 mL overnight culture of *P. aeruginosa* IA1 in L-broth (1% Bacto-Tryptone, 0.5% yeast extract; Difco Chemical Co., St Louis, MO, USA). The OMPs were electroblotted on to an Immobilon P polyvinylidinedifluoride membrane (Millipore, Bedford, MA, USA) which was then dried and stained. The OprG band was excised from the blot and subjected to N-terminal amino acid sequencing by Dr P. Williams (Department of Pharmaceutical Sciences, University of Nottingham, Nottingham, UK). The procedure

```
OprG    MRKSWLTASLLALTVASPFAAADIQGHKAGDFIIRGGFATVDPDDSSSDIKLDGAKQRGT 60
OmpW    MKQTICLAVLAALLAAPVFA------HQEGDFIVRAGIASVVPNDSS-DKVLN--TQ--S 49
        *:::   * * ** .*. **      *: ****:*.*:*:* *:*** *  *:  .*  :

OprG    KATVDSDTQLGLTFTYMFADKWGVELVAATPFNHQVDVKGLGPGLDGKLADIKQLPPTLL 120
OmpW    ELAVNSNTHLGLTLGYMFTDNISFEVLARTPFSHKISTSGGELGSLGDIGETKHLPPTFM 109
           : :*:*:*:****: ***:*: ..*::* ***.*::...*   *  *.:.: *:****::

OprG    LQYYPMGGTNSAFQPYGGLGVNYTTFFDEDLASNRKAQGFSSMKLQDSWGLAGELGFDYM 180
OmpW    VQYY-FGEANSTNRPYVGAGLNYTTFFDESFNSTGTNNALSDLKLDDSWGLAANVGFDYM 168
        :*** :* :**: :** * *:********.: *. . :.:*.:**:******.::*****

OprG    LNEHALFNMAVWYMDIDTKASINGPSALGVNKTKVDVDVDPWVYMIGFGYKF 232
OmpW    LNDSWFLNAYVWYANIETTATYKA----GADAKSTDVEINPWVFIIAGGYKF 216
        **:  ::*  *** :*:*.*: :.    *.: ...**:::***::*. ****
```

Figure. Sequence alignment by Clustal Wb of the deduced amino acid sequence of *P. aeruginosa* OprG and its closest homologue, *V. cholerae* OmpW. The experimentally determined N-terminal amino acid sequence is shown in bold letters, preceded by the deduced signal sequence. The symbols under the sequences represent identity (*) or high (:) or moderate (.) similarity at a given sequence position.

was repeated, yielding identical N-terminal sequences of DIQGHKAGD, and the sequence was used to search a release of *P. aeruginosa* genomic sequences (http://www.pseudomonas.com). This identified a single homologous open reading frame encoding a pre-protein with a predicted 22 amino acid signal sequence, followed by a peptide of 210 amino acids that included the above N-terminal sequence as the first nine amino acids. This sequence (Figure) was found in contig 216 (from bases 51,972 to 51,277 on the complementary strand; 15 September 1998 release of genomic sequences), located at about 1000 kbp on the physical genomic map on macrorestriction fragment *Dpn*I-L, *Spe*I-A (http://www.bit.uq.edu.au/pseudomonas/map.html).

The predicted 210 amino acid protein is 43% identical and 58% similar to a *Vibrio cholerae* OMP, OmpW[6] (Figure), which is itself a member of a family of minor OMPs that are predicted to form eight-stranded β-barrels.[7] Two of the closer homologues, DoxH[8] and AlkL,[9] are encoded in operons involved in naphthalene and alkane catabolic pathways respectively, but their precise functions are not known.

In conclusion, we have identified and sequenced the last of the *P. aeruginosa* major OMPs. This will permit further studies to determine its cellular function and potential role in quinolone uptake.

Acknowledgements

We would like to acknowledge the Pseudomonas Genome Project for the timely release of their sequence data. We acknowledge funding from the Canadian Cystic Fibrosis Foundation and through the Medical Research Council of Canada Distinguished Scientist Award to R.E.W.H. Funding for F.S.L.B. was from a special award from the Canadian Cystic Fibrosis Foundation for genomic studies.

References

1. Hancock, R. E. W., Siehnel, R. & Martin, N. (1990). Outer membrane proteins of *Pseudomonas*. *Molecular Microbiology* **4**, 1069–75.

2. Hancock, R. E. W. & Worobec, E. A. (1998). Outer membrane proteins. In *Biotechnology Handbook*, Vol. 10 (Montie, T., Ed.), p. 139–167. Plenum Press, London.

3. Hancock, R. E. W. & Carey, A. M. (1979). Outer membrane of *Pseudomonas aeruginosa*. Heat- and 2-mercaptoethanol-modifiable proteins. *Journal of Bacteriology* **175**, 6890–1.

4. Yates, J. M., Morris, G. & Brown, M. R. (1989). Effect of iron concentration and growth rate on the expression of protein G in *Pseudomonas aeruginosa*. *FEMS Letters* **49**, 259–62.

5. Chamberland, S., Bayer, A. S., Schollaardt, T., Wong, S. A. & Bryan, L. E. (1989). Characterization of mechanisms of quinolone resistance in *Pseudomonas aeruginosa* strains isolated in vitro and in vivo during experimental endocarditis. *Antimicrobial Agents and Chemotherapy* **33**, 624–34.

6. Jalajakumari, M. B. & Manning, P. A. (1990). Nucleotide sequence of the gene *ompW*, encoding a 22 kDa immunogenic outer membrane protein of *Vibrio cholerae*. *Nucleic Acids Research* **18**, 180.

7. Baldermann, C., Lupas, A., Lubieniecki, J. & Engelhardt, H. (1998). The regulated outer membrane protein omp21 from *Comamonas acidovorans* is identified as a member of a new family of eight-stranded beta-sheet proteins by its sequence and properties. *Journal of Bacteriology* **180**, 3741–9.

8. Denome, S. A., Stanley, D. C., Olson, E. S. & Young, K. D. (1993). Metabolism of dibenzothiophene and naphthalene in *Pseudomonas* strains: complete DNA sequence of an upper naphthalene catabolic pathway. *Journal of Bacteriology* **175**, 6890–1.

9. Van Beilen, J. B., Eggink, G., Enequist, H., Bos, R. & Witholt, B. (1992). DNA sequence determination and functional characterization of the OCT-plasmid-encoded *alkJKL* genes of *Pseudomonas oleovorans*. *Molecular Microbiology* **6**, 3121–36.

Outer membrane permeability of the antibiotic-supersusceptible lipid A mutants of *Escherichia coli* to hydrophobic steroid probes

J Antimicrob Chemother 1999; **43:** 608–610

Patrick Plésiat[a] and Martti Vaara[b,c]*

[a]*Laboratoire de Bactériologie, Faculté de Médecine, Besançon, France;* [b]*Department of Bacteriology and Immunology, University of Helsinki;* [c]*Department of Bacteriology, National Public Health Institute, Helsinki, Finland*

*Correspondence address. Haartmaninkatu 3, 00014 Helsinki, Finland. Tel: +358-9-1912-6302; Fax: +358-9-1912-6382;
E-mail: martti.vaara@helsinki.fi

Sir,

Many clinically important Gram-negative bacteria possess effective outer membrane (OM) permeability barriers that markedly limit the penetration and, thus, the activities of hydrophobic antibiotics, such as erythromycin and rifampicin, and large antibiotic molecules, such as vancomycin.[1,2] This, in part, explains why these pathogens are resistant to most of the recently discovered agents (e.g. the oxazolidinones and everninomicin) that exhibit potent activities against Gram-positive bacteria.[3] The barrier function of the OM relies principally on the compact leaflet of lipopolysaccharide (LPS) molecules that covers the cell surface and which is fairly impermeable to hydrophilic and (moderately) hydrophobic compounds. This is exemplified by the recently described *Escherichia coli* mutants, *lpxA* and *lpxD*, in which the lipid A moieties of the LPS have undergone profound alterations and which are almost as susceptible to hydrophobic antibiotics as the Gram-positive reference strains.[3,4] The MICs of a large number of antibiotics for the

E. coli lpxA mutant are 30- to 1000-fold lower than those for clinical isolates of *E. coli*.[4] Other LPS mutants, such as those in which the inner core polysaccharide moieties of the LPS are defective (Re type), also exhibit antibiotic supersusceptibility, although to a lesser extent than the lipid A mutants.

The most reliable method of quantifying the permeability of the OM to hydrophobic compounds is that developed by Plésiat & Nikaido[5] which allows the rates of penetration of various steroid probes across the lipid portion of the bilayer to be accurately determined. The results are expressed in terms of the permeability coefficient (P) in nanometres per second (nm/s). With this approach, these investigators showed that the OM of a Re mutant of *Salmonella typhimurium* was up to 25- and 16-fold more permeable to uncharged steroids and to testosterone hemisuccinate (a monoanionic probe) respectively than that of the parent strain.[5] P values for the most antibiotic-hypersusceptible strains, i.e. the lipid A mutants, have not yet been reported. In the present study, we demonstrate that the diffusion rate of testosterone hemisuccinate through the *lpxA*-type OM is much higher than that through the Re-type OM.

The strains used in the study were as follows: the lipid A mutants of *E. coli* SM101 (*lpxA)* and *E. coli* CDH23-213 (*lpxD*, the gene formerly known as *omsA*, *firA* and *ssc*), both of which are thermosensitive, i.e. SM101 grows well at 28°C, but not at 37°C, and CDH23-213 grows well at 20°C and 37°C, but not at 42°C; the corresponding isogenic parent-type strains, SM105 ($lpxA^+$) and CDH23-210 ($lpxD^+$), which were used as controls; and the *E. coli* mutant strain, D21f2 (*rfa*), which has a defective inner core oligosaccharide and which elaborates Re-type (heptoseless) LPS. The antibiotic susceptibilities of all of the strains have been characterized previously.[3,4]

The study strains were transformed with the recombinant plasmid, pLE689, that mediates the 3-oxosteroid Δ^1-dehydrogenase enzyme of *Comamonas testosteroni*.[5] The media used were LB agar and broth, both containing 10 g of tryptone (Oxoid, Basingstoke, UK), 5 g of yeast extract (Difco, Detroit, MI, USA) and 5 g of NaCl, at pH 7.2, and supplemented with kanamycin at a concentration of 25 mg/L. The agar plates and broth cultures were incubated at 30°C. The steroid permeability assay was performed as described previously.[6] Briefly, cells in the exponential growth phase were collected by centrifugation at room temperature and resuspended in 50 mM HEPES, pH 7.4, to an A_{650} of 3. Tubes containing 700 μL of bacterial suspension diluted three-, six- or 30-fold in the same buffer were incubated at 30°C with gentle agitation. The neutral steroid, testosterone, or its hemisuccinate derivative was added at time 0 to give final concentrations of 50 μM and 200 μM respectively. Following incubation for 10 min, >95% of the steroid was removed by two brief extractions with ethyl acetate. The concentration of the Δ-dehydrogenated product was then determined by high-performance liquid chromatography (HPLC) and the permeability coefficients were calculated according to an equation based on Ficks' First Law of Diffusion.[6]

Table. P values (nm/s) for testosterone and testosterone hemisuccinate in the lipid A mutants of *E. coli*, *lpxA* and *lpxD*, their isogenic parent-type strains (controls) and a mutant strain of *E. coli*, D21f2, with a defective inner core oligosaccharide

	P	
Strain	testosterone	testosterone hemisuccinate
CDH23-213 (*lpxD*)	3260	890
SM101 (*lpxA*)	4270	1100
CDH23-210 ($lpxD^+$)	60	8
SM105 ($lpxA^+$)	120	20
D21f2 (*rfa*)	3620	170

The P values for testosterone hemisuccinate in the lipid A mutants, SM101 (*lpxA*) and CDH23-213 (*lpxD*), were approximately 900–1100 nm/s—approximately five- to six-fold greater than that in the *E. coli* Re mutant, D21f2 (Table), approximately ten-fold greater than that previously determined in the Re mutant of *S. typhimurium* (data not shown) and approximately 50- to 100-fold greater than those in the control strains (Table). Of considerable note, the high P values for the lipid A mutants demonstrated here are comparable to that (approximately 1000 nm/s) previously determined for the wild-type strain, *S. typhimurium* SL696,[5] following exposure to deacylpolymyxin B (DAPB), a potent permeabilizer of the OM.[7] This suggests that the OM permeability barrier to a representative anionic hydrophobic probe molecule is quantitatively equally defective in both the lipid A mutants and the DAPB-permeabilized cells.

The P values for testosterone in the lipid A mutants (3260 nm/s and 4270 nm/s in CDH23-213 and SM101, respectively) were similar to that (3620 nm/s) in the Re mutant (Table). This suggests that the Re-type OM is already maximally permeable to this neutral hydrophobic probe. In accord with this observation, previous investigators have shown that the P coefficient for another uncharged steroid, androstanedione, in DAPB-treated *S. typhimurium* was comparable to that in the Re mutant of *S. typhimurium*.[5]

Studies such as the one described here add to our knowledge of the properties of the permeability barriers of mutationally defective OMs. This in turn provides information that might help in designing novel antibiotics with activities against Gram-negative bacteria. In the light of increasing antibiotic resistance worldwide, such agents are needed desperately.

Acknowledgement

The excellent technical assistance of Colette Godard is gratefully acknowledged.

References

1. Nikaido, H. (1996). Outer membrane. In *Escherichia coli and Salmonella typhimurium, Cellular and Molecular Biology*, (Neidhardt, F. C., Ed.), pp. 29–47. American Society for Microbiology, Washington, DC.

2. Nikaido, H. & Vaara, M. (1985). Molecular basis of bacterial outer membrane permeability. *Microbiological Reviews* **49**, 1–32.

3. Vaara, M. (1993). Antibiotic-supersusceptible mutants of *Escherichia coli* and *Salmonella typhimurium. Antimicrobial Agents and Chemotherapy* **37**, 2255–60.

4. Vuorio, R. & Vaara, M. (1992). The lipid A biosynthesis mutation *lpxA2* of *Escherichia coli* results in drastic antibiotic supersusceptibility. *Antimicrobial Agents and Chemotherapy* **36**, 826–9.

5. Plésiat, P. & Nikaido, H. (1992). Outer membranes of Gram-negative bacteria are permeable to steroid probes. *Molecular Microbiology* **6**, 1323–33.

6. Plésiat, P., Aires, J. R., Godard, C. & Köhler, T. (1997). Use of steroids to monitor alterations in the outer membrane of *Pseudomonas aeruginosa. Journal of Bacteriology* **179**, 7004–10.

7. Vaara, M. (1992). Agents that increase the permeability of the outer membrane. *Microbiological Reviews* **56**, 395–411.

Evaluation of the Etest for determining the in-vitro susceptibilities of *Prevotella intermedia* isolates to metronidazole

J Antimicrob Chemother 1999; **43:** 610–611

Pierre P. Poulet*, Danièle Duffaut and Jean P. Lodter

Department of Oral Biology, School of Dental Medicine, Paul Sabatier University, Toulouse, France

*Correspondence address. Buccal Biology Laboratory, Faculty of Dentistry, 3 chemin des Maraîchers, 31063 Toulouse cedex, France. Tel: +33-5-62-17-36-08; Fax: +33-5-61-06-51-13.

Sir,

The bacterial composition of the subgingival flora varies widely from individual to individual and from pocket to pocket in the same individual. Certain pathogens, particularly *Prevotella intermedia*, are associated with severe forms of periodontal disease.[1] Not all patients respond equally to periodontal treatment and antibiotics can enhance the effects of surgical intervention in those with rapidly progressive or refractory periodontitis.[2] One of the most frequently prescribed antibiotics is metronidazole, resistance to which is rarely encountered, although treatment with high dosages may be necessary to eradicate some pathogens. In common with the causes of infection at other sites, periodic surveys of the susceptibilities of the aetiological agents of periodontitis are necessary in order to detect changes in the patterns of resistance and thereby to facilitate optimal antibiotic therapy. The present study was undertaken to determine the in-vitro susceptibilities of *P. intermedia* isolates to metronidazole and to evaluate the efficacy of the Etest for this purpose.

Subgingival plaque samples were obtained from patients with periodontitis and inoculated on to solid medium within 3 h; the plates were incubated under anaerobic conditions at 37°C for 3–7 days. Isolates of *P. intermedia* ($n = 13$) were identified according to standard laboratory procedures. Susceptibility to metronidazole was determined by an agar dilution method recommended by the NCCLS[3] and by the Etest method (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. The medium in both cases was Columbia agar (bioMérieux, Marcy l'Etoile, France) supplemented with 10% sheep blood, haemin (5 mg/L) and menadione (0.5 mg/L), and the inoculum for the agar dilution method was 10^5 cfu. *P. intermedia* isolates CIP 103607 and CIP 6322 (Collection of the Pasteur Institute, Paris) were used as controls and all plates were incubated for 48 h at 37°C in an anaerobic chamber.

The mean MIC for the 13 isolates, as determined by the reference agar dilution method, was 0.98 mg/L (range 0.25–2 mg/L), whereas the mean MIC determined by the Etest was 0.82 mg/L (range 0.047–2 mg/L). In 33% of cases, the MICs determined by the two methods were the same. Of the remaining isolates, the MICs for 87% and 93% were within one and two two-fold dilutions, respectively. For only one strain was the difference between the MICs determined by the two methods more than four two-fold dilutions (MIC of 0.047 mg/L with the Etest and 0.25 mg/L with the agar dilution method).

We have shown in the present study that the MICs of metronidazole for clinical isolates of *P. intermedia* are broadly similar, regardless of whether they are determined by the Etest or the agar dilution method; 93% of results differed by no more than two two-fold dilutions. Others have reported similar results, i.e. concordance or a difference of only one or two two-fold dilutions in 34.3%, 74% and 90% of strains, respectively.[4] We conclude that the Etest is a simple, rapid and reliable method of determining the MICs of metronidazole for *P. intermedia* isolates. However, its relatively high cost will preclude it from being used as a routine susceptibility testing method.

The MIC_{100} of metronidazole for the 13 isolates (2 mg/L), based on MICs determined by the agar dilution method, is in agreement with the susceptibility data reported by other

investigators[5,6] and is less than the MIC breakpoint (8 mg/L) recommended by the NCCLS.[3] The highest MIC recorded by us, 2 mg/L, was one two-fold dilution higher than that reported by Sutter *et al.*[6] However, those investigators included isolates other than *P. intermedia* and did not distinguish one black pigmented species from another.

Although only a small number of isolates were evaluated, the present study demonstrates the excellent in-vitro activity of metronidazole against *P. intermedia*, with 100% of the isolates tested being susceptible to this agent on the basis of the recommended MIC breakpoint. This observation is in accord with multicentre studies in the USA which determined the susceptibilities of much larger numbers of isolates, and confirms that the isolation of a strain belonging to this species that exhibits resistance to metronidazole is a very rare event.

Acknowledgements

This study was supported by Rhône–Poulenc Rorer, France. We thank Professor Giorgio Cimasoni, Dental School, University of Geneva, Switzerland, for his valuable criticism of the manuscript, Professor Henry Dabernat, Microbiology Laboratory, Purpan Hospital, Toulouse, France, and N. Mallet for technical assistance.

References

1. van der Weijden, G. A., Timmerman, M. F., Reijerse, E., Wolffe, G. N., van Winkelhoff, A. J. & van der Velden, U. (1994). The prevalence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* in selected subjects with periodontitis. *Journal of Clinical Periodontology* **21**, 583–8.

2. Loesch, W. J., Schmidt, E., Smith, B. A., Morrison, E. C., Caffesse, R. & Hujoel, P. (1991). Metronidazole in periodontitis II: effects upon treatment needs. *Journal of Periodontology* **62**, 247–57.

3. National Committee for Clinical Laboratory Standards. (1993). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria—Third Edition: Approved Standard M11-A3*. NCCLS, Villanova, PA.

4. Citron, D. M., Ostovari, M. I., Karlsson, A. & Goldstein, E. J. C. (1991). Evaluation of the Etest for susceptibility testing of anaerobic bacteria. *Journal of Clinical Microbiology* **29**, 2197–203.

5. Abu Fanas, S. H., Drucker, D. B., Hull, P. S., Reeder, J. C. & Ganguli, L. A. (1991). Identification, and susceptibility to seven antimicrobial agents, of 61 Gram-negative anaerobic rods from periodontal pockets. *Journal of Dentistry* **19**, 46–50.

6. Sutter, V. L., Jones, M. J. & Ghoneim, A. T. M. (1983). Antimicrobial susceptibilities of bacteria associated with periodontal disease. *Antimicrobial Agents and Chemotherapy* **23**, 483–6.

Comparative in-vitro activities of topical antibiotics against conjunctival isolates

J Antimicrob Chemother 1999; **43:** 611–612

Jabed Iqbal[a]*, Mizanur Rahman[b], M. Shahidul Kabir[b] and Ahmed J. Sharif[c]

[a]*Medical Service Department, Square Pharmaceuticals Ltd, Dhaka;* [b]*Department of Microbiology, National Diagnostic Network, Diabetic Association of Bangladesh, Dhaka;* [c]*Department of Ophthalmology, Dhaka Medical College Hospital, Dhaka, Bangladesh*

*Correspondence address. Medical Service Department, Square Pharmaceuticals Ltd, Square Center, 48 Mohakhali CA, Dhaka 1212, Bangladesh. Tel: +880-2-887729; Fax: +880-2-888608/9; E-mail: square@bangla.net

Sir,

The present study was undertaken to evaluate the in-vitro activities of commonly used ophthalmic antibiotic preparations against external ocular pathogens.

The patients were residents of Dhaka City with clinical diagnoses of acute bacterial conjunctivitis who had not received systemic or intra-ocular antibiotics in the preceding 4 weeks. The diagnosis was based on the presence of conjunctival hyperaemia and one or both of a purulent exudate or crusting on the eyelids. Conjunctival swabs were processed at a central laboratory according to standard laboratory techniques and the isolates obtained after overnight culture were identified by standard procedures. Isolates were regarded as pathogens if they met one of the following criteria: Gram-negative bacillus; β-haemolytic streptococcus; *Streptococcus pneumoniae*; $\geqslant$10 colonies of an α-haemolytic streptococcus or *Staphylococcus aureus*; or $\geqslant$100 colonies of a coagulase-negative staphylococcus (CoNS).[1]

The susceptibilities of the isolates were determined by the disc diffusion method according to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).[2] The medium used was Mueller–Hinton agar, supplemented with 5% sheep blood for streptococci, or chocolate agar for *Haemophilus* spp. The following antibiotics were tested: tetracycline (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), trimethoprim/sulphamethoxazole (25 μg), penicillin (10 μg), erythromycin (15 μg), ciprofloxacin (30 μg), lomefloxacin (30 μg), bacitracin (10 μg), tobramycin (10 μg) and neomycin (30 μg). All of the discs were obtained from Oxoid, Unimed Co. Ltd (UK) and the isolates were categorized as susceptible or resistant according to inhibition zone diameter breakpoints recommended by the NCCLS.[2]

Ninety-eight presumed pathogens were recovered. *S. aureus* was the most common isolate (accounting for 62%

Table. Susceptibilities of 98 external ocular pathogens to various antibiotics available as topical formulations

Bacterium (*n*)	Percentage of susceptible isolates										
	tetracycline	chloramphenicol	gentamicin	trimethoprim/ sulphamethoxazole	penicillin	erythromycin	ciprofloxacin	lomefloxacin	bacitracin	tobramycin	neomycin
S. aureus (61)	52	74	86	71	12	39	84	80	91	81	80
CoNS (24)	50	57	100	71	8	62	79	80	85	87	62
S. pneumoniae (8)	71	100	29	100	75	100	25	25	88	12	12
Haemophilus influenzae (4)	100	100	80	25	25	25	75	75	25	50	25
Pseudomonas aeruginosa (1)	0	0	100	0	NT	NT	100	100	0	100	100
All isolates (98)	55	72	82	69	16[a]	49[a]	78	74	86	76	68

NT, not tested.
[a]Of 97 isolates tested.

of the pathogens), followed by CoNS (24%), although the roles of these organisms as causes of acute conjunctivitis are controversial. The susceptibilities of the isolates are summarized in the Table. The ranking of the antibiotics in terms of percentage susceptible strains (in descending order) was as follows: bacitracin; gentamicin; ciprofloxacin; tobramycin; lomefloxacin; chloramphenicol; trimethoprim/sulphamethoxazole; neomycin; tetracycline; erythromycin; and penicillin. While chloramphenicol is the most commonly used ophthalmic antibiotic in Bangladesh, followed by ciprofloxacin and gentamicin (S. Hossain, personal communication), it was active against fewer isolates than the latter two drugs. Ciprofloxacin was active against a marginally higher percentage of isolates than lomefloxacin and, of the aminoglycosides tested, gentamicin was active against the highest percentage of strains.

The results of this study are in accord with published data from Pakistan[3] which also showed that bacitracin was the most active antibiotic tested (91% of isolates being susceptible); moreover, the percentages of strains that were susceptible to tetracycline, chloramphenicol, gentamicin and neomycin were comparable to those reported here. Whilst the absence of comprehensive published data concerning the antibiotic susceptibilities of ocular pathogens precludes drawing accurate conclusions about trends in susceptibility/resistance, the results of this limited study suggest that no currently available topical formulation is active *in vitro* against all of the common pathogens. Only one antibiotic combination, trimethoprim/sulphamethoxazole, was evaluated by us and this agent was not superior to the single antibiotics tested. In conclusion, we have demonstrated that bacitracin is the most broad-spectrum agent currently available in Bangladesh as empirical treatment of patients with external ocular infections.

Acknowledgements

We are grateful to the Diabetic Association of Bangladesh for providing financial support for this study. We would also like to thank Mr Shazeed Hossain of Square Pharmaceuticals Ltd, Dhaka, for providing the unpublished data referred to in this article.

References

1. Jacobsen, J. A., Call, N. B., Kasworm, E. M., Dirks, M. S. & Turner, R. B. (1988). Safety and efficacy of topical norfloxacin versus tobramycin in the treatment of external ocular infections. *Antimicrobial Agents and Chemotherapy* **32**, 1820–4.

2. National Committee for Clinical Laboratory Standards. (1993). *Performance Standards for Antimicrobial Disc Susceptibility Tests—Fifth Edition: Approved Standard M2-A5*. NCCLS, Villanova, PA.

3. Woodland, R. M., Darougar, S., Thaker, U., Cornell, L., Siddique, M., Wania, J. *et al*. (1992). Causes of conjuctivitis and keratoconjuctivitis in Karachi, Pakistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, 317–20.