Effect of Salicylic Acid on the Membrane Proteome and Virulence of *Pseudomonas aeruginosa*

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**PURPOSE.** This study aimed to determine the effect of salicylic acid on the membrane proteome, sensitivity to antibiotics, and production of microbial keratitis by *Pseudomonas aeruginosa*.

**METHODS.** *P. aeruginosa* 6294 was grown in the presence or absence of 30 mM salicylic acid. Bacterial membrane proteins were extracted in carbonate buffer, separated using two-dimensional gel electrophoresis and identified by mass spectrometry. The minimum inhibitory concentration (MIC) of various antibiotics was determined using *P. aeruginosa* 6294 grown in presence or absence of salicylic acid. The scratch mouse model of microbial keratitis was used to determine whether treatment with 30 mM salicylic acid could improve the outcome of infection.

**RESULTS.** Growth in salicylic acid altered the membrane proteome of *P. aeruginosa* 6294. Eighteen proteins, including OprE, OprD, MexA, OprG, PilQ, and flagellin-type A protein, were downregulated, six proteins, including OprM and OprB, were upregulated, and nine proteins were unaffected by growth in salicylic acid. Growth in salicylic acid slightly increased the resistance to carbapenem antibiotics but did not affect MICs of the other antibiotics tested. Salicylic acid treatment significantly reduced the clinical score of eyes and bacterial load in eyes during microbial keratitis but had no effect on numbers of infiltrating neutrophils.

**CONCLUSION.** Salicylic acid altered the membrane proteins of *P. aeruginosa*, slightly increased the resistance of the bacterium to carbapenem antibiotics only, and was able to reduce the pathogenicity associated with *P. aeruginosa* infection of mouse corneas. Salicylic acid may be useful as an antimicrobial agent in the treatment of *Pseudomonas* keratitis.

Keywords: salicylic acid, membrane proteome, *Pseudomonas aeruginosa*, keratitis

*Pseudomonas aeruginosa* is an opportunistic pathogen and remains the most common cause of infection of the cornea for those people who wear contact lenses.1 Annualized incidence rates of microbial keratitis vary in studies, although overall they are in good agreement, with rates during extended wear (i.e., people sleep in lenses) being approximately 20 per 10,000 wearers per year.2 One of the problems associated with ocular infections with *P. aeruginosa* remains the most common cause of infection of the cornea for those people who wear contact lenses.1 Annualized incidence rates of microbial keratitis vary in studies, although overall they are in good agreement, with rates during extended wear (i.e., people sleep in lenses) being approximately 20 per 10,000 wearers per year.2 One of the problems associated with ocular infections with *P. aeruginosa* is the increasing rate of resistance to antibiotics being reported worldwide.1,3 Multidrug resistance efflux systems enable *P. aeruginosa* to protect itself by detoxifying and extruding different toxic compounds including antibiotics.4 Overproduction of multiple drug efflux systems (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) in *P. aeruginosa* provides increased levels of tolerance to organic solvents and clinically useful antibiotics.5 The MexAB-OprM efflux pump system is probably the most important of these resistance strategies, being responsible for resistance to various antibiotics and chemotherapeutic agents, including quinolones and β lactams.6,7 The MexCD-OprJ and MexEF-OprN systems are less significant than MexAB-OprM, and normally remain silent in the presence of functioning MexAB-OprM and various gene repressors. However, if the gene repressors are mutated (which can happen in the presence of various antibiotics), these other efflux pumps can mediate resistance to, for example, fluoroquinolones, aminoglycosides, and certain β lactams.8–12 Another outer membrane protein involved in the antibacterial resistance of *P. aeruginosa* is OprD.13 In this case lack of the protein mediates resistance to cephalosporins.

There are many known or predicted outer membrane proteins (OMPs), including membrane-bound porins, in the genome of *P. aeruginosa* PAO1.14 Porins have a β barrel transmembrane structure and allow access of substances such as sugars to the cell.15,16 Porins can contribute to the production and release of proinflammatory cytokines and cause cell injury and apoptosis.17,18 *P. aeruginosa* contains different membrane-associated secretory apparati involved in the release of a large number of extracellular proteins, including virulence factors, to the external environment.19 Different secretion systems are involved in release of alkaline protease (type I); elastase (type II); the cytotoxins ExoU, ExoS, ExoT, and ExoY (type III); the CdrA adhesin (type V); and Tsc2 toxin (type VI).20 In addition, several other surface-associated virulence structures, such as pili, flagella, lipopolysaccharide, and capsules, are also attached to the cell via the outer membrane and are exposed to the external environment.

*P. aeruginosa* can become resistant to many clinically relevant antibiotics, so there is a need to investigate...
alternative agents for controlling the diseases caused by this bacterium. Salicylic acid can reduce the attachment of \textit{P. aeruginosa} to human corneal epithelial cells in vitro\textsuperscript{21} and downregulates a number of virulence factors in \textit{P. aeruginosa}\textsuperscript{22,23}, even at concentrations that do not greatly affect growth. However, salicylic acid is known to induce resistance of \textit{P. aeruginosa} to imipenem via downregulation of OprD, but does not affect sensitivity to chloramphenicol, norfloxacin, tetracycline, or cepfiroxime.\textsuperscript{24} Salicylic acid can increase the permeability of the outer membrane of \textit{P. aeruginosa} to the \(\beta\)-lactam nitrocefin.\textsuperscript{25} For the related bacterium, \textit{Burkholderia cepacia}, salicylic acid induced resistance to ciprofloxacin, chloramphenicol, and trimethoprim, but not to ceftazidime.\textsuperscript{26} Growth in salicylic acid of \textit{Escherichia coli} induces reversible resistance to chloramphenicol, ampicillin, tetracycline, and nalidixic acid.\textsuperscript{27} If salicylic acid is to be a useful antibacterial agent, or adjunct agent to be used in conjunction with traditional antibiotic therapy in the treatment of disease, a more detailed understanding of its action on the bacterium, resistance development, and treatment in animal models is needed.

\section*{Materials and Methods}

\subsection*{Bacterial Strains and Growth Conditions}

\textit{Pseudomonas aeruginosa} 6294, isolated from a case of microbial keratitis,\textsuperscript{28} was subcultured in 10 mL trypticase soy broth (Oxoid, Ltd., Sydney, Australia) at 37°C overnight. The cultures were grown to stationary phase without agitation and bacterial cells harvested by centrifugation (3000 \(\times\) 10 minutes, 20°C), washed twice, and suspended in PBS at a concentration of 1 \(\times\) 10\(^7\) colony-forming units (cfu)/mL (OD of 0.1 at 660 nm).

\subsection*{Isolation of OMPs and Two-Dimensional–PAGE}

The bacteria were added 1:10 to 400 mL either fresh trypticase soy broth (TSB) alone (control) or fresh TSB with a sub–minimum inhibitory concentration (MIC) of salicylic acid and incubated for 20 hours. The cells were harvested by centrifugation at 3000g for 10 minutes at 20°C and washed twice in PBS. The cell pellets were snap-frozen at –80°C and freeze-dried. Outer membrane proteins were prepared by the modified sodium carbonate extraction method as previously described by Nouwens et al.\textsuperscript{29} and freeze-dried. Outer membrane proteins were acquired from two separate cultures and analyzed separately to ensure reproducibility.

Freeze-dried OMPs were solubilized in 2 mL isoelectric focusing (IEF) sample buffer (tetradecanoylamine-propyl-dimethyl ammonioipropylcarbonate-sulfonate-14 1% wt/vol Calibiochem-Novabiochem Co., San Diego, CA, USA; tributylphosphine [TBP; BioRad, Gladsmuir, NSW, Australia] 2 mL; urea [BDH, Tingalpa, QLD, Australia] 7 M; Thiourea [Sigma, Castle Hill, NSW, Australia] 2 M; carrier ampholytes [Bio-Rad] 0.5% wt/vol; bromophenol blue). The sample mixtures were vortexed for 1 minute and centrifuged (20,000g, 10 minutes, 4°C) to remove any insoluble matter. Samples containing 250 \(\mu\)g protein were loaded onto 17 cm pH 4-7 immobilized pH gradient (IPG; Bio-Rad) dry strips by rehydration overnight at 18°C. Isolelectric focusing and subsequent SDS-gel electrophoresis were conducted as described by Nouwens et al.\textsuperscript{29,30} Protein spots were imaged and digitized using a PerkinElmer ProXPress Proteomic Imaging System (Melbourne, VIC, Australia). Two-dimensional electrophoresis was performed in triplicate for each sample preparation; thus, using the duplicate OMP preparations, six gels were generated for analysis.

Differently expressed proteins were defined as those showing an increase or decrease in spot intensity of greater than 2-fold over the control on average from the six gels. PDQuest (Bio-Rad) software was used for the comparative analyses, spot quantitation, gel comparison, and data analysis of digitized gel images. Thirty-five gel spots showing reproducible changes in abundance, and 12 spots that did not change, were selected for further analysis. Each protein spot was identified by searches against the \textit{Pseudomonas} genome sequencing project (www.pseudomonas.com) using the MACOT tool (www.matrixscience.com). For convincing spot identification, % sequence coverage, number of matching peptide molecular masses, and total protein mass and PI were used. Further confidence in the identification was provided by mass spectrometry/mass spectrometry sequencing of the strongest eight peptide signals generated in the first mass spectrometry (MS) scan.

\subsection*{Determination of Effect of Salicylic Acid on the MIC of Various Antibiotics}

The method used followed that outlined by the Clinical and Laboratory Standards Institute (document M7-A7; ISBN 1-56238-587-9) using the microdilution broth test, with the following modifications. Bacteria were routinely grown in the presence of 30 mM salicylic acid or without salicylic acid and the following antibiotics: ceftazidime (32–0.25 \(\mu\)g/mL), tetracycline (32–0.25 \(\mu\)g/mL), ciprofloxacin (2–0.015 \(\mu\)g/mL), imipenem (32–0.25 \(\mu\)g/mL), meropenem (8–0.125 \(\mu\)g/mL), and erythromycin (640–10 \(\mu\)g/mL). The MIC was determined as the lowest concentration of the antibiotic that inhibited growth of the bacteria after incubation at 37°C for 18 hours. The MIC of bacteria grown in the presence or absence of salicylic acid was recorded and all experiments were performed on at least two different occasions.

\subsection*{Treatment of Microbial Keratitis With Salicylic Acid in an Animal Model}

The animal model used has been described previously.\textsuperscript{31} A total of 12 AJ mice (The Jackson Laboratories, Bar Harbor, ME, USA) (in two sets of 6) were used according to the guidelines put forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and institutional ethics approval as obtained. Briefly, after anesthesia, the right cornea of inbred 6- to 8-week-old male mice was incised and 10 \(\mu\)L containing 5 \(\times\) 10\(^8\) cfu \textit{P. aeruginosa} 6294, previously grown in TSB (without salicylic acid) was applied directly onto the wounded cornea.

After 18 hours, mouse eyes were treated hourly for the next 5 hours with 30 mM salicylic acid in PBS or vehicle alone. Mice were monitored with slit-lamp by a masked observer at 24 hours after bacterial infection. Each of five parameters (exudate, epithelial defect, corneal infiltrate extent, corneal opacity, and corneal edema,) was graded on a scale of 0 (none) to 4 (severe) during slit-lamp examination. The parameter grades were summed to produce a single slit-lamp examination score ranging from 0 (normal eye) to a theoretical maximum of 20. After mice were euthanized, corneas were collected and analyzed for bacterial numbers and numbers of infiltrating PMNs. Bacteria were quantified after growth on trypticase soy agar (Oxoid) for 18 hours at 37°C.\textsuperscript{32} Numbers of PMNs in corneas were calculated based on the amount of myeloperoxidase in corneal homogenates.\textsuperscript{31,32} Results were expressed as mean \(\pm\) standard deviation (SD) and mean of the PMN per cornea \(\pm\) SD. Results were analyzed by independent \(t\)-test for comparisons between groups.
RESULTS

Effect of Salicylic Acid on the Membrane Proteome of P. aeruginosa 6294

Most OMPs of P. aeruginosa were resolved within the pH range from 4 to 6 and a molecular mass ranging from 18 to 45 kDa (Fig. 1) as shown previously. A total of 47 protein spots, 35 spots that reproducibly changed on growth in salicylic acid and 12 spots that remained unchanged, were isolated and identified by matrix-assisted laser desorption/ionization-time of flight-MS following in-gel tryptic digest. Most membrane proteins characterized by peptide-mass mapping in P. aeruginosa stain 6294 were matched to open reading frame sequences from P. aeruginosa POA1 database. Most of the identified spots were verified as OMP or had unknown functions (Table 1). Thirty-five of the 47 spots identified were seen to change in abundance between treatments. Most protein spots were present in lower abundance in salicylic acid–treated samples when compared with the control (Table 1). The following proteins were downregulated: OprF, OprD, MexA, OprG, OprL, protein derived from PA1041 (probable OMP), protein derived from PA2113 (opdO; probable porin), fimbrial protein precursor, PilQ, flagellin type-A, HsiC2, peptidyl-prolyl cis-trans isomerase, ComL, protein derived from PA2800 (VacJ), aconitate hydratase-1, LptE and one hypothetical protein (from PA1324). The following proteins were upregulated: OprB, protein derived from PA2291 (opbA; probable porin), protein derived from PA0162 (opdC; probable protein), OprM, protein derived from PA2837 (opmA; probable OMP), and D-ala-D-ala-carboxypeptidase. Twelve spots representing nine proteins were unaffected by growth in presence of salicylic acid (Table 2), including PagL, Opr86, and OprH.

Effect of Salicylic Acid on Sensitivity of P. aeruginosa to Antibiotics

Table 3 gives details of the MIC of bacteria grown in presence or absence of salicylic acid. Only with imipenem or meropenem was there any increase MIC when the bacteria were grown in the presence of salicylic acid. Although the MIC for meropenem was increased, the bacteria were still considered to be sensitive to this antibiotic when grown in presence of salicylic acid (Table 3).

Effect of Salicylic Acid on Production of Microbial Keratitis by P. aeruginosa 6294

Figure 2 shows the results of treatment with salicylic acid on the microbial keratitis produced by P. aeruginosa 6294. There was a significant decrease in overall clinical (slit-lamp) score (P = 0.005) and number of bacteria remaining in the corneas 24 hours after initiation of infection (P = 0.04). However, the numbers of PMNs infiltrating the corneas at 24 hours after infection was not affected (P > 0.05) by treatment with salicylic acid.

DISCUSSION

This study has demonstrated that growth of P. aeruginosa in the presence of a subinhibitory concentration of salicylic acid alters the membrane proteome of the bacterium, has a slight effect of sensitivity to carbapenem antibiotics, and can reduce the pathogenicity associated with infection of the cornea. Using the same strain and culture conditions, we have previously shown that the same salicylic acid treatment can prevent adhesion of P. aeruginosa to surfaces, reduce the twitching and swimming motility of this bacterium, and reduce the production of proteases and quorum-sensing molecules. The ability of salicylic acid to reduce adhesion of P. aeruginosa to surfaces and reduce forms of motility might be related to the finding in the current study that salicylic acid reduced the production of two fimbrial proteins, including PilQ and flagellin type-A protein. The PilQ is involved in extruding type IV pilus fibers through the bacterial outer membrane and thereby facilitating twitching motility, whereas flagella are involved in direct binding to epithelial mem-

![Figure 1. Two-dimensional gel electrophoresis of membrane proteome of P. aeruginosa 6294. (A) Proteins extracted from cells grown in TSB alone. (B) Proteins extracted from cells grown in TSB + 30 mM salicylic acid. Numbers refer to proteins identified in Table 1.](https://example.com/image1.png)
Another cell surface protein, OprF, which was reduced after growth in salicylic acid, has also been shown to have a role in adhesion to epithelial cells. 37,38 Mutation of oprF reduces secretion of type 3 secretion system effectors ExoS and ExoT, as well as the virulence factors pyocyanin, exotoxin A, lectin PA-1L, and elastase, and the quorum-sensing signal molecules N-(3-oxododecanoyl)-L-homoserine lactone and N-butanoyl-L-homoserine lactone. 38 Salicylic acid can inhibit production of pyocyanin, protease, elastase, acylated homoserine lactones, and corneal epithelial cell invasion or cytotoxicity,22,23 and the current study also demonstrated downregulation in the presence of salicylic acid of LptE, a protein involved in the assembly of lipopolysaccharide in the outer membrane,39 and HsiC2, a protein of the type VI secretion system 40 that is involved in the secretion of the Tse2 toxin.20 As many of these virulence factors have been

### Table 1. Outer Membrane Proteins of *P. aeruginosa* Strain 6294 That Showed Greater Than 2-Fold Differences in Abundance After Growth in Salicylic Acid

<table>
<thead>
<tr>
<th>Spot Identification No., See Fig. 1</th>
<th><em>P. aeruginosa</em> Gene No.</th>
<th>Gene/Protein Name</th>
<th>Sequence Coverage, %/No. of Peptides Identified</th>
<th>TMr, kDa/TpI</th>
<th>Change (Average Magnitude of Difference)</th>
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<tbody>
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<td></td>
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<tr>
<td>25 P. aeruginosa oprF/OprF</td>
<td>23/7</td>
<td>37.6/4.98</td>
<td>Downregulated (2×)</td>
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<td></td>
</tr>
<tr>
<td>27 P. aeruginosa oprD/OprD</td>
<td>25/8</td>
<td>48.3/4.96</td>
<td>Downregulated (4×)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 P. aeruginosa mexA/MexA</td>
<td>20/6</td>
<td>40.9/8.75</td>
<td>Downregulated (3×)</td>
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<td></td>
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<tr>
<td>31 P. aeruginosa oprG/OprG</td>
<td>34/5</td>
<td>25.2/4.85</td>
<td>Downregulated (3×)</td>
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</tr>
<tr>
<td>36 P. aeruginosa oprL/OprL</td>
<td>29/5</td>
<td>17.9/6.32</td>
<td>Downregulated (2×)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 P. aeruginosa oprD/OprD</td>
<td>41/15</td>
<td>44.5/5.7</td>
<td>Downregulated (3×)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 P. aeruginosa oprL/OprL</td>
<td>23/7</td>
<td>21.7/5.30</td>
<td>Downregulated (2×)</td>
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<tr>
<td>35 P. aeruginosa oprD/OprD</td>
<td>31/12</td>
<td>50.7/5.48</td>
<td>Upregulated (2×)</td>
<td></td>
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<tr>
<td>37 P. aeruginosa oprL/OprL</td>
<td>42/15</td>
<td>48.8/4.97</td>
<td>Upregulated (2×)</td>
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<td>41 P. aeruginosa oprD/OprD</td>
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<td>51.9/5.59</td>
<td>Upregulated (4×)</td>
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<td>42 P. aeruginosa oprD/OprD</td>
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<td>50.5/4.54</td>
<td>Upregulated (2×)</td>
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<td>43 P. aeruginosa oprD/OprD</td>
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<td>44 P. aeruginosa oprD/OprD</td>
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<td><strong>Pili or flagella proteins</strong></td>
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<td>4 no code (not in PAOI genome)</td>
<td>44/5</td>
<td>15.8/5.52</td>
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<td>26.8/5.09</td>
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<td>41 P. aeruginosa comL/Competence protein ComL</td>
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<td>28.6/4.98</td>
<td>Downregulated (3×)</td>
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<td>42 P. aeruginosa aceA/Aconitate hydratase-1</td>
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<td>99.1/5.43</td>
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<td>28 P. aeruginosa VacF*</td>
<td>40/7</td>
<td>26.1/5.41</td>
<td>Downregulated (2×)</td>
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<tr>
<td>24 P. aeruginosa lptE/LptE</td>
<td>57/10</td>
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<td>38 P. aeruginosa dacC/D-mena-D-mena-carboxypeptidase</td>
<td>17/6</td>
<td>42.4/6.27</td>
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Proteins were identified by MALDI/TOF-MS peptide-mass matching. *Pseudomonas aeruginosa* gene no. refers to the genome annotation (www.pseudomonas.com). TMr and TpI, theoretical molecular mass (kDa) and isoelectric point.

* Alternative name for gene derived from other bacterial types (www.pseudomonas.com). To be classified as upregulated or downregulated, proteins had to have at least a 2-fold change in abundance.
shown to be important during infection of the cornea.\textsuperscript{41–45} we tested the ability of salicylic acid to reduce the virulence of \textit{P. aeruginosa} 6294 in a murine keratitis model. Salicylic acid significantly reduced the numbers of bacteria that were present in the corneas of mice 24 hours after infection and reduced the clinical score. Interestingly, salicylic acid did not affect the number of PMNs in the corneas. Salicylic acid is a nonsteroidal anti-inflammatory drug and so can inhibit arachidonic acid metabolism. Also, salicylate has been reported to inhibit superoxide generation by neutrophils,\textsuperscript{46} but does not inhibit their chemotactic response in vitro.\textsuperscript{47} Based on these findings, it appears that a likely explanation of the effectiveness of salicylic acid on neutrophil function such as generation of superoxide or phagocytosis of bacteria cannot be ruled out. It should be noted that \textit{P. aeruginosa} was grown in vitro in the presence of salicylic acid for 20 hours but in vivo were exposed to the salicylic acid for only 5 hours. Thus, there is the possibility that changes seen during growth in vitro may not necessarily be reflected in vivo due to the differences in exposure time of the bacteria.

Other membrane proteins were also changed by growth in presence of salicylic acid. These included such proteins as MexA, OprM, OprD, OprG, OprL, and OprB. The most important resistance efflux transporter systems in \textit{P. aeruginosa} are the resistance-nodulation-division (RND) super family,\textsuperscript{4} which is composed of the \textit{mexA, mexB, oprM} gene operon in all strains of \textit{P. aeruginosa}. Following inactivation of one of the RND super family (\textit{mexA, mexB, or oprM} genes), strains become much more sensitive to antibiotics.\textsuperscript{48} The current study showed that growth in salicylic acid reduced the production of MexA, which anchors the RND complex to the inner membrane via fatty acids and acts as a membrane bridge protein for the MexB and OprM subunits.\textsuperscript{49} The reduction in MexA might lead to an increase in sensitivity of antibiotics such as the carbapenems.\textsuperscript{50} On the other hand, growth in salicylic acid increased expression of OprM, which might be expected to increase resistance to carbapenam antibiotics.\textsuperscript{50} There was a small increase in resistance to imipenem and meropenem after growth in presence of salicylic acid (Table 3). However, as the MexA/OprM changes were opposite of each other, it is perhaps more likely that this increase in carbapenem resistance was due to reduced expression of OprD.\textsuperscript{13,51} especially as there was no concomitant increase in resistance to quinolones.\textsuperscript{7}

Unlike the case when \textit{Burkholderia cepacia} or \textit{E. coli} was grown in salicylic acid,\textsuperscript{26,27} \textit{P. aeruginosa} did not have an increase in resistance to ciprofloxacin or tetracycline. The nonspecific sugar diffusion porin OprB was upregulated in response to salicylic acid. OprB is a general carbohydrate transport protein that is selective for passage across the outer membrane of sugars, including mannitol, fructose, and glycerol, and plays a central role in carbohydrate uptake.\textsuperscript{52} The OprG is a relatively unexplored protein in the \textit{P. aeruginosa} proteome, but might be involved in cytotoxic responses to epithelial cells.\textsuperscript{53} The OprG does not contribute to complement resistance or biofilm formation, but is involved in the cytotoxic responses of strain PA14, a known cytotoxic strain of \textit{P. aeruginosa}.\textsuperscript{55} Although the strain used in the current study, \textit{P. aeruginosa} 6294, is an invasive rather than cytotoxic strain,\textsuperscript{28,44} invasive strains have been shown to produce cytotoxic responses to human corneal epithelial cells in vitro,\textsuperscript{54} and so the reduction in OprG may have resulted in reduced pathology due to reduction in cytotoxic responses in vivo response to salicylic acid. The OprG was downregulated by growth in salicylic acid. The OprG is required to maintain outer membrane integrity of \textit{E. coli}\textsuperscript{55} and fluidity of \textit{P. aeruginosa} outer membrane.\textsuperscript{56} The OprL is decreased after exposure of \textit{P. aeruginosa} to tobramycin or ciprofloxacin.\textsuperscript{57}
In conclusion, this study has shown that salicylic acid can affect the membrane proteome of *P. aeruginosa* and down-regulate the expression of many OMPs. Changes to these proteins can manifest as a small increase in resistance to carbapenem antibiotics but salicylic acid treatment can result in a dramatic decrease in the ability of *P. aeruginosa* to infect corneas. The data imply that salicylic acid may be an appropriate cotreatment choice with fluoroquinolone antibiotics for treatment of *P. aeruginosa* keratitis.

**Acknowledgments**

Supported in part by a postgraduate research scholarship from the Vision CRC, and by the Federal Australian Government through the CRC scheme.

**Disclosure:** M. Bandara, None; P. Sankaridurg, None; H. Zhu, None; E. Hume, None; M. Willcox, Alcon Laboratories (C, R), Allergan, Inc. (C, R), Bausch + Lomb (C, R), Johnson and Johnson Vision Care (C, R), Ophthecs Corp. (C, R), Coopervision (C, R)

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