CLINICAL AND EXPERIMENTAL
OPTOMETRY

REVIEW

Overview of mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*: an ocular perspective

Clin Exp Optom 2018; 101: 162–171 DOI:10.1111/cxo.12621

Dinesh Subedi MSc (Microbiology) BSc
Ajay Kumar Vijay PhD (Optometry) BSc
Mark Willcox DSc PhD FAAO FBCLA FASM FARVO
School of Optometry and Vision Science, Faculty of Science, The University of New South Wales, Sydney, New South Wales, Australia
E-mail: d.subedi@unsw.edu.au

Submitted: 8 May 2017
Revised: 18 June 2017
Accepted for publication: 19 June 2017

Treatment of *Pseudomonas aeruginosa* eye infections often becomes a challenge due to the ability of this bacterium to be resistant to antibiotics via intrinsic and acquired mechanisms. Transfer of resistance due to interchangeable genetic elements is an important mechanism for the rapid transfer of antibiotic resistance in this pathogen. As a result, drug-resistant strains are becoming increasingly prevalent worldwide. This review systematically analyses data from recent publications to describe the global prevalence and antibiotic sensitivity of ocular *P. aeruginosa*. Thirty-seven studies were selected for review from PubMed-based searches using the criteria 'microbial keratitis OR eye infection AND *Pseudomonas aeruginosa* AND antibiotic resistance' and limiting to papers from 2011 onward, to demonstrate the antibiotic resistance from isolates from around the world. Subsequently, we reviewed the ways in which *P. aeruginosa* can become resistant to antibiotics. Both the rate of isolation of bacteria in general (79 per cent of cases), and prevalence of *P. aeruginosa* (68 per cent of all isolates) were highest in contact lens-related microbial keratitis. The average resistance rate to common ocular antibiotics such as ciprofloxacin (9 per cent), gentamicin (22 per cent) and ceftazidime (13 per cent) remained relatively low. However, there were large variations in resistance rates reported in studies from different countries, for example resistance to ciprofloxacin reached up to 33 per cent. We next reviewed the types of mobile genetic elements (MGEs) such as plasmids, integrons and transposons that are frequently associated with drug resistance in *P. aeruginosa*. MGEs are important for the transmission of resistance to beta-lactams and aminoglycosides and recently have been shown to be potential factors for the transmission of fluoroquinolone resistance. Studies on the molecular mechanisms of resistance transfer in ocular *P. aeruginosa* have begun to be reported and will provide valuable information on the emergence of new antibiotic resistance and potential to treat resistant strains.

Key words: antibiotic resistance, keratitis, *Pseudomonas aeruginosa*

The opportunistic pathogen *Pseudomonas aeruginosa* is well-known for its remarkable adaptation to different environments. With diverse metabolic pathways and a large repertoire of pathogenic mechanisms, this Gram-negative bacillus is able to survive and grow under a broad range of environmental conditions, including but not limited to: on medical equipment,[1] in water systems,[2] in ventilators[3] and in the presence of some disinfectants.[4]

Diseases associated with this bacterium range from mild folliculitis to life-threatening pneumonia and septicaemia.[5] Predisposing factors for *Pseudomonas* infections include a compromised immune system and impaired anatomical structures caused by, among other things, burns, cystic fibrosis and mechanical abrasions such as wearing of contact lenses.[6,7] *P. aeruginosa* is one of the most common pathogens causing vision-threatening eye infections.[7–10] Pre-existing contact lens wear, ocular surface disease, ocular trauma and prior ocular surgery are risk factors for developing *Pseudomonas* keratitis.[7,11]

Reduced susceptibility to antibiotics and disinfectants has been widely reported in clinical isolates of *P. aeruginosa*,[12–14] which makes infection with this bacterium difficult to treat. *P. aeruginosa* is not only naturally immune to many antimicrobials due to chromosomally encoded resistance genes,[15,16] but also has the capacity to acquire mobile genetic elements (MGEs).[15,16] This latter mechanism is important in emergence and rapid spread of drug resistance. As in systemic *Pseudomonas* infections,[17,18] the rate of isolation of antibiotic-resistant *P. aeruginosa* has been increasing in ocular infections.[12,13] However, very few reports describe the mechanism of resistance of ocular isolates.

Antibiotic resistance genes can originate from both pathogenic and non-pathogenic bacteria. Bacteria acquire the genes in order to survive in unfavourable conditions such as in presence of antibiotics. Plasmids and other MGEs play a vital role in this phenomenon. They act as vectors for both resistance gene capture and dissemination. This paper reviews the latest prevalence and antibiotic resistance patterns of *P. aeruginosa* isolated from eye infections

Source (references)	Rate of isolation of bacteria (%)	Prevalence of *P. aeruginosa* (%)
Acute conjunctivitis[25]	NA	19.4
Dacryocystitis[26]	60.8	9.7
Endophthalmitis[27–31]	45.5	11.6
Lacrimal duct obstruction[32]	72.0	5.0
Microbial keratitis[33–49]	63.3	18.2
Contact-lens related microbial keratitis[19,20]	78.9	68.2
Neonatal keratitis[50]	74.3	41.0
Paediatric keratitis[51,52]	65.4	20.4
Other eye infections[48,53–55]	31.7	13.8

NA: data not available.

Table 1. Average rate of isolation of bacteria and average prevalence of *Pseudomonas aeruginosa* in various eye infections

and evaluates the MGE-mediated resistance to the most important anti-pseudomonas drug classes: beta-lactams, aminoglycosides and fluoroquinolones.

P. AERUGINOSA IN EYE INFECTIONS

Data on the prevalence and antibiotic sensitivity of ocular isolates of *P. aeruginosa* were collected from publications from 2011 to 2016. The literature was obtained from PubMed using the search keywords 'microbial keratitis OR eye infection AND *Pseudomonas aeruginosa* AND antibiotic resistance'. Only those articles were included that reported on isolates from the eye and for which antimicrobial resistance had been determined.

Publications with irrelevant research questions, non-English full text and/or those lacking quantitative information on resistance and sensitivity were excluded. Out of a total of 62 articles, 37 were relevant to the scope of this review. Therefore, data of those 37 papers were analysed and are summarised in this section of the review.

The rate of isolation of bacteria from ocular infections was highest in contact lens-related microbial keratitis compared to other eye infections.[19,20] Furthermore, the prevalence of *P. aeruginosa* was much higher in contact lens-related microbial keratitis[19,20] compared to other eye infections (Table 1). A report from Australia showed culture positivity rates of 65 per cent from microbial keratitis and *P. aeruginosa* was the most common isolate.[11] However, obtaining a positive culture from clinical samples depends on many factors: antibiotic treatment before collection of sample, appropriate culture conditions, ability of infecting microbes to grow artificially, robustness of inflammatory response, and involvement of agents other than bacteria such as viruses and protozoa, and these may be the reasons for lower culture rates in other studies.[21–24]

The literature covered data from 17 countries (Table 2), and a higher culture positive rate was noted in the Netherlands (78.9 per cent) which also had the highest prevalence of *P. aeruginosa* (68.2 per cent).[19] The reason for the latter finding is probably due to the study examining only cases of microbial keratitis. A relatively high prevalence was observed in a study from Pakistan[13] perhaps because of selection of isolates only from contact lenses.

The overall antibiotic sensitivity pattern of *P. aeruginosa* was determined by compiling information on the total number of isolates tested for individual antibiotics and the number of resistant or susceptible isolates to each antibiotic. Ciprofloxacin was the most frequently tested antibiotic, followed by gentamicin and ceftazidime. Levofloxacin was the most effective antibiotic for ocular isolates of *P. aeruginosa* followed by ciprofloxacin and amikacin with respective sensitivities of 94.6, 90.9 and 90.2 per cent (Figure 1).

The sensitivity rate varied greatly between countries. For example, studies from Ethiopia (66.7 per cent),[26] China (50 per cent),[28] Pakistan (40 per cent),[13] Germany (53 per cent)[46] and Mexico (12 per cent)[38] had much lower sensitivity rates to gentamicin than the overall average (78.5 per cent). *P. aeruginosa* isolated from other body site infections often showed much higher resistance than eye isolates. In separate studies, isolates of *P. aeruginosa* from urinary tract infection,[56] multiple body sites of intensive care unit patients[57] and cystic fibrosis cases[58] showed only 62, 41 and 44 per cent sensitivity to ciprofloxacin, respectively.

The rate of antibiotic resistance observed in this study is close to results that were reported before 2010.[22,59] This suggests that rate of antibiotic resistance in ocular *P. aeruginosa* is stable. Furthermore, reports of antibiotic resistance monitoring studies have shown that the antibiotic resistance pattern in eye isolates of *P. aeruginosa* has not substantially changed over this period.[53,54,60] However, similar to the finding of this analysis, antibiotic resistance rate was higher in certain geographical locations.[61] This trend is probably related to the extensive use of antibiotics in systemic infections and agriculture, over-the-counter availability and improper diagnosis and prophylaxis.[62]

In contrast, a 10-year retrospective study of *P. aeruginosa* isolated from pneumonia showed that resistance to antibiotics has significantly increased over the past decade.[63] Although the resistance rate was relatively low and stable in eye isolates of *P. aeruginosa*, there is the possibility of transfer of resistance between eye and non-eye isolates, thus leading to infections with resistance isolates. Therefore, it is necessary to monitor the mechanism of resistance transfer in eye *P. aeruginosa*.

MOBILE GENETIC ELEMENTS-ASSOCIATED DRUG RESISTANCE IN *P. AERUGINOSA*

Literature on MGE-associated drug resistance in *P. aeruginosa* was searched in PubMed, MEDLINE and Google. The search was performed using key terms '*Pseudomonas aeruginosa*', AND 'mobile genetic elements' OR 'plasmid' OR 'integrons' OR 'transposons', AND 'antibiotic resistance', with a refinement of AND 'eye infection' after initial searches. Reference lists of all the related literature were further manually checked for relevant information on drug resistance and associated

Country (references)	Average rate of isolation of bacteria from eye infections (%)	Average prevalence of *P. aeruginosa* (%)
Netherlands[19]	78.9	68.2
India[30,35,37,40,43,50]	76.3	22.7
Ethiopia[26,36]	71.9	25.7
USA[27,29,34,49,53,54,57]	66.7	14.5
New Zealand[45]	65.5	3.4
China[28,51]	59.6	9
Germany[32,46]	57.5	7.5
Taiwan[31,39,52,56]	53.1	27.5
Mexico[38]	37.6	12
Morocco[55]	36.4	12.5
Thailand[48]	27	NA
Pakistan[13]	NA	44
Singapore[42]	NA	32
UK[44]	NA	24.3
Georgia[25]	NA	19.4
USA[41]	NA	16.2
Saudi Arabia[33]	NA	6

NA: data not available.

Table 2. Average rate of isolation of bacteria and average prevalence of *Pseudomonas aeruginosa* from ocular infections in various countries

MGEs. The collected data were used to describe the MGE-associated drug resistance in *P. aeruginosa*.

Plasmids are circular double-stranded DNA molecules auxiliary to chromosomal DNA found in many, if not all bacterial types. They are diverse in size and function. Some just carry one to two genes but others may harbour as many as 400 genes.[64] Plasmids not only exist independently of chromosomal DNA but also multiply autonomously,[65] which means several copies can be contained in one bacterial cell. They do not take part in basic cellular functions such as cell growth and multiplication; rather they often carry genetic elements that help the bacteria to survive and grow in unfavourable environmental conditions such as in the presence of lethal antibiotics.

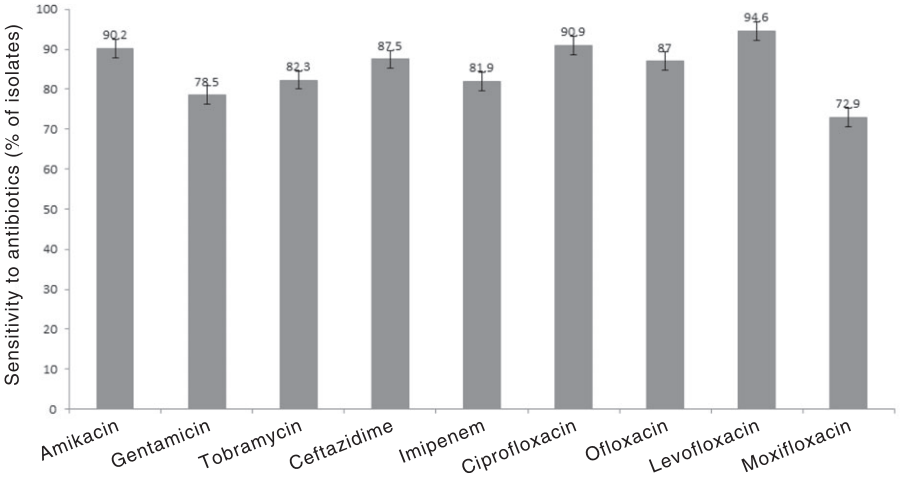

Figure 1. Global antibiotic sensitivity of ocular isolates of *Pseudomonas aeruginosa* (n = 3,064), data pooled from recent studies.[13,19,20,25–55] (Error bars correspond to standard error of the mean).

Plasmids can encode enzymes that confer resistance to different antimicrobial substances such as antibiotics, disinfectants, heavy metals and even UV radiation.[66] Furthermore, plasmids can move with or without captured chromosomal genes from one bacterial cell to another, in a method called horizontal gene transfer.[65] If plasmids carry antibiotic resistance genes, the recipient bacteria will be able to express antibiotic resistance.

Transposons and integrons are other genetic elements that are as important as plasmids for the horizontal transfer of bacterial resistance and are common in clinical strains of *P. aeruginosa*.[16] Commonly called jumping genes, transposons can undergo transposition to move themselves from one location to another within the same or different DNA molecules, for example plasmids or the chromosome.[64,67] Their size varies from as short as 1.5 kilobase pairs (kb) to as long as 26.6 kb, and they consist of an insertion sequence and at least one open reading frame.[16] The former encodes the transposase that facilitates their movement and the latter might contain gene(s) for antibiotic resistance.[16] On the other hand, integrons are gene capture systems that capture gene cassettes, including but not limited to, antibiotic resistance genes and aid their expression.[68] Integrons (Figure 2) can be located in plasmids or transposons.[69]

The presence of MGEs in *P. aeruginosa* is significantly associated the multidrug resistance patterns.[71,72] About 30–40 per cent of multidrug-resistant *P. aeruginosa* isolates have been recorded to carry one or more plasmids.[71,73,74] Variations in carriage rates of as low as 14 per cent[75] to as high as 100 per cent[76] have been reported. Moreover, the occurrence of plasmids is associated with both the nature of antibiotics and the source of isolation of the bacteria. For example, beta-lactam-resistant strains carry a higher frequency of plasmids than strains resistant to other classes of antibiotics.[73,77] Similarly, plasmid size is generally larger in

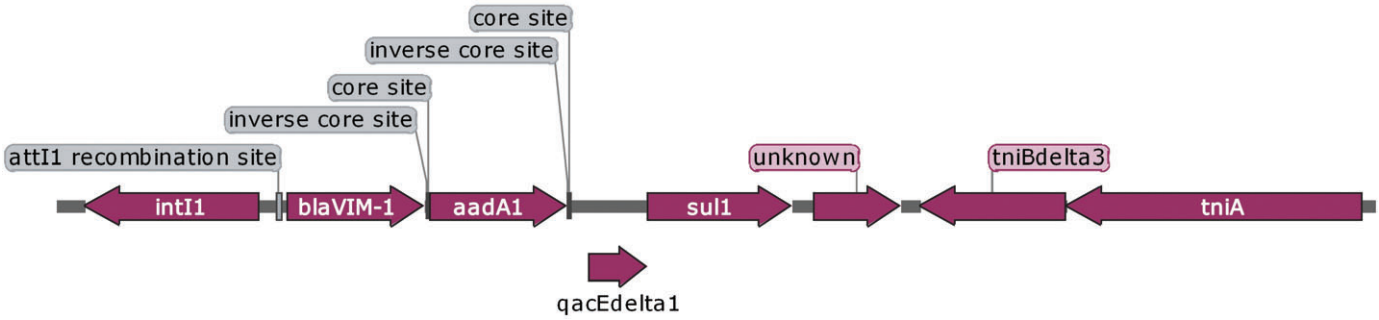

Figure 2. An integron (7,621 bp) of *Pseudomonas aeruginosa* plasmid PAMBL-2 (GenBank accession no. GQ422828[70]). The genes within the integron are: intI1 = integrase, attI = attachment gene for integron, blaVIM-1 = metallo-beta-lactamase, aadA1 = aminoglycoside adenylyltransferase, QacEdelta1 = multidrug exporter, and sul1 = dihydropteroate synthase, unknown = unknown gene, tniBdelta3 = partial sequence of putative transposase, tniA = putative transposase.

strains showing aminoglycoside resistance.[77] Furthermore, integron-associated antibiotic resistance genes have been identified in several disease outbreaks.[78–81] Reports from varying geographic locations showed about one-half of the total multidrug resistance *P. aeruginosa* strains carry integrons.[82–84]

Resistance traits associated with MGEs indicate that they are most probably recently acquired because one strain can horizontally acquire plasmid DNA from other strains.[66] Knowledge of MGE-associated drug resistance is important to monitor resistance behaviour among eye isolates. In addition, this knowledge can be used to develop a plan to reduce antimicrobial resistance and to keep the current antibiotics effective for longer periods.

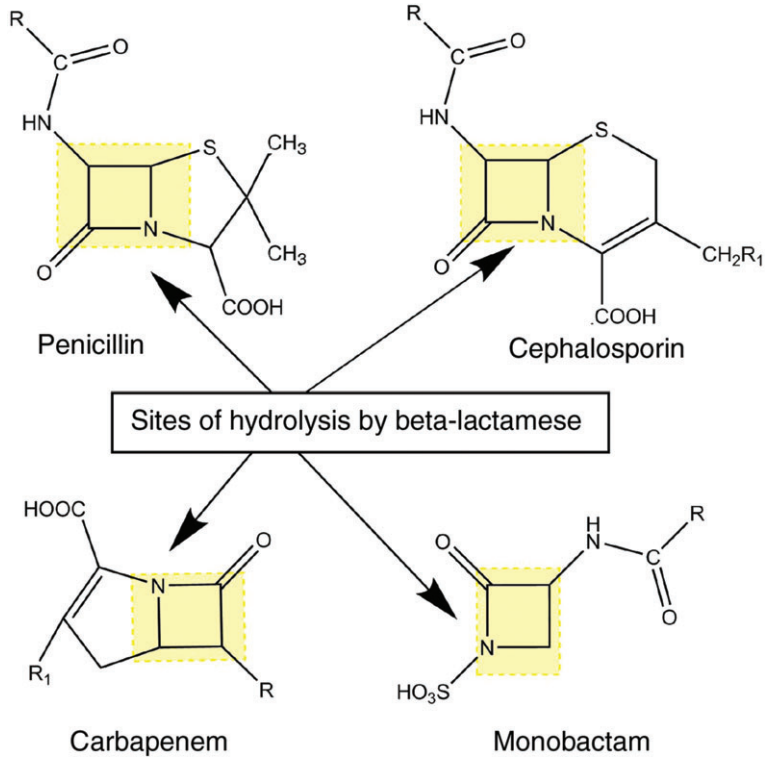

Figure 3. The sites of hydrolysis by beta-lactamase of four important beta-lactam groups

Treatment of microbial eye infections relies mostly on antibiotics from three classes, beta-lactams, aminoglycosides and fluoroquinolones.[85–87] Third generation cephalosporins, gentamicin and ciprofloxacin are the primary choices for treatment of *Pseudomonal* eye infections.[88] Emergence of MGE-related antibiotic resistance in *P. aeruginosa* outside of eyes[71–74,77] suggests that similar patterns may be present in ocular isolates.

BETA-LACTAM RESISTANCE

Beta-lactam antibiotics are the most widely used therapeutic choice for treatment of bacterial infections, accounting for 60 per cent of total antibiotics used by weight.[89] Their high effectivity, chemical diversity, comparatively low cost with minimum side-effects make this class of antibiotics popular in treatment of microbial infections.[90] Cephalosporins such as cephazolin are commonly used beta-lactams in the treatment of eye infections.[12] They are often prescribed in combination with aminoglycosides as fortified preparations.[85] However, increased beta-lactam resistance in clinical isolates has threatened their therapeutic value.[89] Bacteria become resistant to beta-lactams by producing heterogeneous enzymes commonly called beta-lactamases.[89] These hydrolyse the beta-lactam bond (Figure 3), which is vital for antimicrobial activity, and render the antibiotics ineffective.[91] The genes encoding beta-lactamase can reside on the chromosome or on MGEs (for example, plasmids). Plasmid-encoded enzymes are often expressed constitutively.[91] However, for

Group	Molecular class*	Characteristic	Types that are mediated by MGEs in *Pseudomonas aeruginosa*
1	C	Serine at active site Inducible enzymes Overproduction due to mutation Hydrolysis of cephalosporins Resistance to inactivation by clavulanate and tazobactam	CMY
2	A and D	Serine at active site Broad spectrum penicillinase Inactivation by clavulanate and tazobactam	TEM, SHV, CTX-M, PER, VEB, GES, PSE, KPC, OXA
3	B	Metallo-beta-lactamase Require Zn for activation Lack of inhibition by tazobactam and clavulanate Effective against all beta-lactams except monobactam Inhibition by EDTA	IMP, VIM, GIM, and SMP

*Ambler classification on the basis of amino acid sequences[99].
CMY: active on cephamycins, CTX-M: active on cefotaxime, first isolated at Munich, GES: Guiana-extended spectrum, GIM: German imipenemase, IMP: active on imipenem, KPC: *Kleibsiella pneumoniae* carbapenemase, MGE: mobile genetic elements, OXA: active on oxacillin, PER: *Pseudomonas* extended resistant, PSE: *Pseudomonas*-specific enzyme, SHV: sulfhydryl reagent variable, SMP: Sao Paulo metallo-β-lactamase, TEM: named after the patient (Temoneira), VEB: Vietnam extended-spectrum β-lactamase, VIM: Verona integron-encoded metallo-β-lactamase.

Table 3. Beta-lactamase classification[93]

chromosomally mediated resistance, expression is inducible.[91,92]

A classification by Bush and Jacoby, on the basis of the action of enzymes and susceptibility to beta-lactamase inhibitors, stratifies beta-lactamases into three groups (Table 3) and several sub-groups (not shown here).[93] Group 1 beta-lactamases are chromosome-based cephalosporinase (AmpC) and these are responsible for a low basal level of intrinsic resistance in *P. aeruginosa*.[94] However, its production can be induced by exposure to beta-lactams.[94] Increased production of AmpC due to mutation, coupled with efflux pumps or reduced permeability to beta lactams, may be responsible for resistance against almost all beta-lactams including carbapenems, which are otherwise resistant to inactivation by AmpC.[95] Plasmid-mediated AmpC was first identified in the 1980s in Gram-negative bacteria.[96] However, unlike in *Enterobacteriaceae,* very few reports describe plasmid AmpC in *P. aeruginosa*. A report from India showed 20 per cent of burn isolates of *P. aeruginosa* were phenotypically positive to AmpC beta-lactamase[97] and plasmid-mediated AmpC has been recently detected in clinically isolated *P. aeruginosa* from China.[98]

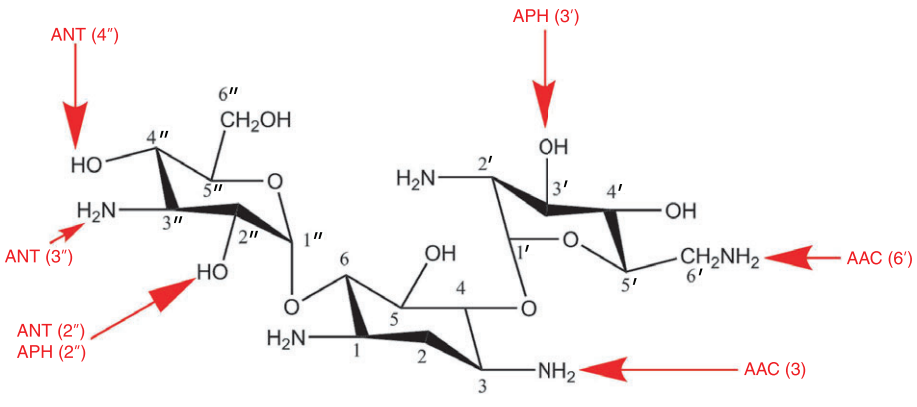

Figure 4. General structure of aminoglycoside and sites of action by aminoglycoside modifying enzymes. AAC: aminoglycoside acetyltransferase, ANT: aminoglycoside nucleotidyltransferase, APH: aminoglycoside phosphoryltransferase.

Group 2 beta-lactamases are a complex group including many families of enzymes that are encoded by genes that are transmissible. This group of enzymes includes both molecular classes A and D (Table 3) that can be inhibited by beta-lactamase inhibitors.[93] The sulfhydryl reagent variable (SHV) family of group 2 beta-lactamase was derived from the chromosome of *Klebsiella* spp. but later incorporated into plasmids.[95] It can hydrolyse broad-spectrum penicillins such as ampicillin and piperacillin but not oxyimino-substituted cephalosporins (ceftazidime and cefotaxime).[95] Variants of SHV (SHV-1, SHV-2, SHV-2[a], SHV-5, SHV-12) have been isolated from *P. aeruginosa*.[95,100,101] The TEM (Temoneira) type beta-lactamases are functionally similar to SHV and were first identified in *Escherichia coli*. Later, many TEM variants (TEM-1, TEM-2, TEM-4, TEM-21, TEM-24, TEM-42, TEM-116) were detected in *P. aeruginosa* with different enzymatic capacities.[93,95,102]

CTX (cefotaxime) represents a new family of beta-lactamases, which are not related to the SHV or TEM types, that has the capacity to hydrolyse cefotaxime. They are also derived from the *Enterobacteriaceae* and were disseminated to other bacteria by horizontal gene transfer.[95] Major variants found in *P. aeruginosa* include CTX-M-1, CTX-M-2, CTX-M-43.[95] Beta-lactamases with the capacity to hydrolyse oxacillin are known as OXA and are predominantly isolated from *P. aeruginosa*. Several variants of OXA enzymes with extended spectrum have been identified in *P. aeruginosa*.[102]

Other extended spectrum beta-lactamases (ESBL) include PER (hydrolysed penicillins and cephalosporins), GES (hydrolysed penicillins and extended-spectrum cephalosporins) and KPC (hydrolysed carbapenems).[93] Many variants of

AMEs	Type (variants)	Resistant to
Aminoglycoside acetyltransferase (AAC)	AAC(6′)-I	Tobramycin, netilmicin, kanamycin, amikacin
	AAC(6′)-II*	Tobramycin, netilmicin, kanamycin, gentamicin
	AAC(3)-I [Ia,Ib,Ic]	Gentamicin
	AAC(3)-II	Gentamicin, tobramycin, netilmicin
	AAC(3)-III	Gentamicin, tobramycin
	AAC(3)-IV	Gentamicin, tobramycin, netilmicin
Aminoglycoside nucleotidyltransferase (ANT)	ANT(2″)-I†	Gentamicin, tobramycin
	ANT(3″)-I	Tobramycin, netilmicin, amikacin
	ANT(4″)-II [IIa, IIb]	Amikacin, tobramycin, isepamicin
Aminoglycoside phosphoryltransferase (APH)	APH(2″)-I	Gentamicin, tobramycin, amikacin
	APH(3′)-I	Kanamycin, neomycin
	APH(3′)-II	Kanamycin, neomycin, gentamicin
	APH(3′)-IV	Kanamycin, neomycin, amikacin

*Most common AAC of *P. aeruginosa.*
†Most common ANT in *P. aeruginosa.*

Table 4. Enzymes frequently reported in *Pseudomonas aeruginosa* responsible for aminoglycosides resistance

these have been detected in *P. aeruginosa* and they have the capacity to generate a high level of resistance to beta-lactams.[102]

Group 3 beta-lactamases, metallo-beta-lactamases, are unique due to the presence of zinc at their active site. They are plasmid-mediated and highly transferable;[103] however, chromosomal integration is also common.[95] The first metallo-beta-lactamase (IMP-1) in *P. aeruginosa* was detected in Japan[103] and since then the incidence of these agents has been increasing.[104,105] Other MGE-associated metallo-beta-lactamase families include SPM, VIM and GIM.[106] These also have been detected in *P. aeruginosa* and their prevalence rates are gradually increasing.[89] Since mutations in the ESBL genes produce variants of enzymes with different specificities for substrates or susceptibilities to beta-lactam inhibitors, the numbers of variants are expanding.

Studies have reported the prevalence of beta-lactam resistance genes in ocular clinical isolates of *P. aeruginosa*, and this ranges from seven per cent to more than 75 per cent.[107–109] TEM, SHV, OX, and CTX-M have been found in the chromosome of an ocular *P. aeruginosa*.[110] In addition, Murugan et al. found integration of TEM-1B, PAO, OXA-50 and VIM-2 in the chromosome of multidrug-resistant *P. aeruginosa* strain from a keratitis patient.[111] There has been no evidence for the plasmid association of these genes in ocular isolates.

AMINOGLYCOSIDE RESISTANCE

Despite the appearance of resistance as far back as the 1960s[112] and high toxicity to eukaryotic cells, aminoglycosides (amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin and tobramycin) have been used for many years for the treatment of *Pseudomonas* infections.[113] Currently, resistance to aminoglycosides in *P. aeruginosa* is very common and is reported from all around the world.[114]

Although aminoglycoside resistance in *P. aeruginosa* may be due to a combination of factors such as a decrease in cell permeability, increased efflux and changes in ribosomes, the most important mechanism is the enzymatic alteration of the active chemical groups in the antibiotics by aminoglycoside-modifying enzymes (AME).[115] These enzymes can modify specific amino and glycoside groups of the molecule vital for the activity of aminoglycosides.[115] According to the types of reactions and the functional groups they attack, three forms of such enzymes have been described (Figure 4): aminoglycoside phosphoryltransferase (APH), aminoglycoside nucleotidyltransferase (ANT) and aminoglycoside acetyltransferase (AAC).[114]

Various protein sequences have been found within each type that are related to different resistance profiles. Accordingly, each class is further sub-classified into different types and are designated by numbers, Roman numerals and letters (Table 4). For example, AAC(6′) acts by acetylation of amino group at 6′ of aminoglycosides; I, II, III … denote a particular resistance profile; and a, b, c … denote unique protein designations. Thus, AAC(3)-Ia and AAC(3)-Ib have the same resistance profile but vary in their amino acid composition.[115]

A functional similarity has been found between AMEs produced by pathogenic Gram-negative bacteria and aminoglycoside-producing *Actinomycetes*.[116] This suggests that aminoglycoside resistance genes originated from organisms that naturally produce aminoglycosides. These genes are often carried on plasmids and/or MGEs, allowing them to disseminate rapidly among bacteria.[117] Furthermore, the MGEs that carry genes for these enzymes have often been associated with the genetic determinants for resistance to other related or unrelated antibiotics.[114,118]

A common gentamicin resistance acetyltransferase gene of *P. aeruginosa*, *aac(3)* is associated with a chromosomally located transposon (Tn801) which also carries a beta-lactamase gene ($bla_{\text{TEM-21}}$).[119] Furthermore, in a burn isolate of *P. aeruginosa*, *aac(3)-I* was located in an integron which also contained other aminoglycoside resistance genes.[120] Similarly, the gene *aph(3′)* which confers resistance to kanamycin, neomycin and streptomycin and *ant(3″)* which confers resistance to streptomycin and gentamicin were carried on MGEs that were associated with beta-lactamase resistance.[121,122]

More recently, a whole genome sequence of multidrug-resistant *P. aeruginosa* from a urine sample demonstrated that this isolate harboured a class 1 integron (In113) carrying *aac(6′)-Iae* and $bla_{\text{IMP-1}}$, which are responsible for high levels of resistance to aminoglycosides and beta-lactams, respectively.[123]

The prevalence of AMEs in aminoglycoside-resistant *P. aeruginosa* has been reported to be as high as 75 per cent.[124] In a separate study of Gram-negative bacteria including *P. aeruginosa*, 94 per cent of isolates carried AMEs coding genes in plasmids.[125] Many new variants of these enzymes have been detected in MGEs of *P. aeruginosa* in non-ocular isolates.[126–128] However, similar studies in ocular isolates are scarce. A variety of AME genes such as *aadA1*, *aph(3′)-IIb*, *aph(3′)-I*, *aph(6)-Id* and

aac(3)-Id have been reported in one multidrug-resistant ocular *P. aeruginosa,*[111] but no other reports have been published.

FLUOROQUINOLONE RESISTANCE

The fluoroquinolones target DNA topoisomerases of bacteria and are broad-spectrum antibiotics. They have gained popularity in ophthalmology to treat ocular bacterial infections and can be used as a monotherapy instead of combinational therapy.[129,130] In particular, ciprofloxacin is a common treatment regimen for microbial keratitis associated with contact lens wear.[11,85] Due to its extensive use, continuous accumulation of resistance in various clinical isolates is being reported worldwide. The rate of ciprofloxacin resistance in clinical isolates of *P. aeruginosa* is more than 20 per cent in the USA.[131] Although the rate of fluoroquinolone resistance in eye isolates of *P. aeruginosa* is lower than those of other systemic infections, a high level of ciprofloxacin resistance has been reported in certain regions of the world.[26,37,42] Ciprofloxacin resistance up to 31 per cent has been reported in ocular *Pseudomonas* species from India and the rate of resistance has been increasing over time.[61]

Resistance to fluoroquinolones developed in bacteria by three different mechanisms: mutations that alter the drug target sites (DNA gyrase and topoisomerase IV), changes that reduce the permeability of the membrane, and transfer of plasmids that carry resistance genes.[15,129,131] Mutations in quinolone-resistance determining regions (QRDRs) of the genes for topoisomerase II and IV are a major cause of resistance to fluoroquinolones of medically important isolates of *P. aeruginosa.*[132] Plasmid-mediated quinolone resistance was first identified in a clinical isolate of *Klebsiella pneumoniae* and the responsible gene was *qnr.*[133] The Qnr protein can bind to DNA gyrase and topoisomerase IV and protects them from inhibition by fluoroquinolones.[134]

Jacoby et al. reported that plasmids also carry genes that encode for beta-lactamases, one of the possible reasons for the occurrence of a high level of fluoroquinolone resistance in ESBL-producing bacteria.[131] Many variants of *qnr* genes (*qnrA*, *qnrB*, *qnrC*, *qnrS* and *qnrD*) have been isolated from Gram-negative bacteria.[135] Plasmid-mediated quinolone resistance has been found in environmental *P. putida* and found to be transferable to *E. coli.*[136] To date, no evidence of the presence of plasmid-mediated *qnr* has been found in *P. aeruginosa.*[135] Various mutations in QRDRs were reported as the cause of resistance to fluoroquinolone[137] in an ocular isolate, but further studies are needed to elucidate acquired fluoroquinolone resistance in ocular *P. aeruginosa.*

CONCLUSION

While ocular *P. aeruginosa* infections can usually be effectively treated with antibiotics from three classes (beta-lactam, aminoglycoside and fluoroquinolones), *P. aeruginosa* can develop resistance against members of these important antibiotic classes in general infections. Genetic mutation and inter/intra-species transfer of MGEs are responsible for the emergence and rapid spread of resistance.

Excessive use of antibiotics in clinical treatments and in agricultural fields has been noted as an important cause for emergence of resistance in bacteria.[62] Moreover, if resistance genes appear in a species, they are not limited to that one species, but can be transferred to other species. This creates a threat of a global pandemic of resistant ocular isolates. Increasing drug resistance in *P. aeruginosa* creates an immediate therapeutic threat because drug-resistant isolates are more difficult to treat[138] and resolution of disease is slower.[139] Analysis of plasmids and related MGEs will help understand the origin of resistance in strains. It is interesting to note that MGEs can contain genes, such as *qacEdelta1* (Figure 1), related to resistance to disinfectants such as polyquaternary ammonium compounds that are used in contact lens disinfecting systems. These genes are common in multiple antibiotic-resistant strains of *P. aeruginosa,*[140] although possession of these types of genes does not necessarily result in increase in resistance to disinfectants.[140] However, possession of *qac* genes in ocular staphylococci does correlate to reduced sensitivity to disinfecting solutions. It will be interesting in future studies to examine relationships between *qac* genes and antibiotic genes to determine whether the exposure of *P. aeruginosa* to disinfectants as might occur during daily wear of contact lenses contributes to the dissemination of antibiotic-resistant genes.

Knowledge of resistance mechanisms is essential to prevent or slow the rate of spreading of resistance in bacteria, either by temporally changing the antibiotic regimen or replacing the antibiotic for which bacteria have mechanisms for dissemination.

REFERENCES

1. Fazeli H, Akbari R, Moghim S, Narimani T, Arabestani MR, Ghoddousi AR. *Pseudomonas aeruginosa* infections in patients, hospital means, and personnel's specimens. *J Res Med Sci* 2012; 17: 332–337.
2. Gad GF, El-Domany RA, Zaki S, Ashour HM. Characterization of *Pseudomonas aeruginosa* isolated from clinical and environmental samples in Minia, Egypt: prevalence, antibiogram and resistance mechanisms. *J Antimicrob Chemother* 2007; 60: 1010–1017.
3. Iversen BG, Jacobsen T, Eriksen HM et al. An outbreak of *Pseudomonas aeruginosa* infection caused by contaminated mouth swabs. *Clin Infect Dis* 2007; 44: 794–801.
4. Loughlin MF, Jones MV, Lambert PA. *Pseudomonas aeruginosa* cells adapted to benzalkonium chloride show resistance to other membrane-active agents but not to clinically relevant antibiotics. *J Antimicrob Chemother* 2002; 49: 631–639.
5. Yahr TL, Parsek MR. *Pseudomonas aeruginosa.* Prokaryotes: A Handbook on the Biology of Bacteria, Vol 6, 3rd edn, 2006; pp 704–713.
6. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. *Crit Care Med* 1999; 27: 887–892.
7. Stapleton F, Carnt N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. *Eye (Lond)* 2012; 26: 185–193.
8. Sy A, Srinivasan M, Mascarenhas J et al. Pseudomonas aeruginosa keratitis: outcomes and response to corticosteroid treatment. *Invest Ophthalmol Vis Sci* 2012; 53: 267–272.
9. Weed MC, Rogers GM, Kitzmann AS, Goins KM, Wagoner MD. Vision loss after contact lens-related *Pseudomonas* keratitis. In. http://www.EyeRounds.org/cases/171-pseudomonas-keratitis.htm: EyeRounds.org, 2013.
10. Fleiszig SM, Evans DJ. The pathogenesis of bacterial keratitis: studies with *Pseudomonas aeruginosa. Clin Exp Optom* 2002; 85: 271–278.
11. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea* 2008; 27: 22–27.
12. Willcox MD. Review of resistance of ocular isolates of *Pseudomonas aeruginosa* and staphylococci from keratitis to ciprofloxacin, gentamicin and cephalosporins. *Clin Exp Optom* 2011; 94: 161–168.
13. Abidi SH, Sherwani SK, Siddiqui TR, Bashir A, Kazmi SU. Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. *BMC Ophthalmol* 2013; 13: 57.
14. Mohammadinia M, Rahmani S, Eslami G et al. Contact lens disinfecting solutions antibacterial efficacy: comparison between clinical isolates and the standard ISO ATCC strains of

Pseudomonas aeruginosa and *Staphylococcus aureus*. *Eye (Lond)* 2012; 26: 327–330.
15. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? *Clin Infect Dis* 2002; 34: 634–640.
16. Kung VL, Ozer EA, Hauser AR. The accessory genome of *Pseudomonas aeruginosa*. *Microbiol Mol Biol Rev* 2010; 74: 621–641.
17. .Shah DA, Wasim S, Essa Abdullah F. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from urine samples of Urinary Tract Infections patients in Karachi, Pakistan. *Pak J Med Sci* 2015; 31: 341–345.
18. Senthamarai S. Resistance pattern of *Pseudomonas aeruginosa* in a tertiary care hospital of Kanchipuram, Tamilnadu, India. *J Clin Diagn Res* 2014; 8: DC30–32.
19. Hoddenbach JG, Boekhoorn SS, Wubbels R, Vreugdenhil W, Van Rooij J, Geerards AJ. Clinical presentation and morbidity of contact lens-associated microbial keratitis: a retrospective study. *Graefes Arch Clin Exp Ophthalmol* 2014; 252: 299–306.
20. Mohammadpour M, Mohajernezhadfard Z, Khodabande A, Vahedi P. Antibiotic susceptibility patterns of *Pseudomonas* corneal ulcers in contact lens wearers. *Middle East Afr J Ophthalmol* 2011; 18: 228–231.
21. Schaefer F, Bruttin O, Zografos L, Guex-Crosier Y. Bacterial keratitis: a prospective clinical and microbiological study. *Br J Ophthalmol* 2001; 85: 842–847.
22. Sueke H, Kaye S, Neal T et al. Minimum inhibitory concentrations of standard and novel antimicrobials for isolates from bacterial keratitis. *Invest Ophthalmol Vis Sci* 2010; 51: 2519–2524.
23. Fong CF, Hu FR, Tseng CH, Wang IJ, Chen WL, Hou YC. Antibiotic susceptibility of bacterial isolates from bacterial keratitis cases in a university hospital in Taiwan. *Am J Ophthalmol* 2007; 144: 682–689.
24. Begum NN, Al-Khattaf AS, Al-Mansouri SM, Yeboah EA, Kambal AM. A study of bacterial isolates from corneal specimens and their antibiotic resistance profile. *Saudi Med J* 2006; 27: 41–45.
25. Chikviladze D, Nikuradze N, Gachechiladze K, Miqeladze M, Metreveli D. Microbial structure of acute bacterial conjunctivitis. *Georgian Med News* 2013: 12–15.
26. Assefa Y, Moges F, Endris M et al. Bacteriological profile and drug susceptibility patterns in dacryocystitis patients attending Gondar University Teaching Hospital, Northwest Ethiopia. *BMC Ophthalmol* 2015; 15: 34.
27. Sridhar J, Kuriyan AE, Flynn HW Jr, Miller D. Endophthalmitis caused by *Pseudomonas aeruginosa*: Clinical features, antibiotic susceptibilities, and treatment outcomes. *Retina* 2015; 35: 1101–1106.
28. Long C, Liu B, Xu C, Jing Y, Yuan Z, Lin X. Causative organisms of post-traumatic endophthalmitis: a 20-year retrospective study. *BMC Ophthalmol* 2014; 14: 34.
29. Schimel AM, Miller D, Flynn HW Jr. Endophthalmitis isolates and antibiotic susceptibilities: a 10-year review of culture-proven cases. *Am J Ophthalmol* 2013; 156: 50–52.e51.
30. Pathengay A, Moreker MR, Puthussery R et al. Clinical and microbiologic review of culture-proven endophthalmitis caused by multidrug-resistant bacteria in patients seen at a tertiary eye care center in southern India. *Retina* 2011; 31: 1806–1811.
31. Chen KJ, Sun MH, Lai CC et al. Endophthalmitis caused by *Pseudomonas aeruginosa* in Taiwan. *Retina* 2011; 31: 1193–1198.
32. Prokosch V, Prokosch JE, Promesberger J et al. Bacterial spectrum and antimicrobial susceptibility patterns in acquired and connatal lacrimal duct stenosis. *Curr Eye Res* 2014; 39: 1069–1075.
33. Al-Dhaheri HS, Al-Tamimi MD, Khandekar RB, Khan M, Stone DU. Ocular pathogens and antibiotic sensitivity in bacterial keratitis isolates at King Khaled eye specialist hospital, 2011 to 2014. *Cornea* 2016; 35: 789–794.
34. Borkar DS, Acharya NR, Leong C et al. Cytotoxic clinical isolates of *Pseudomonas aeruginosa* identified during the Steroids for Corneal Ulcers Trial show elevated resistance to fluoroquinolones. *BMC Ophthalmol* 2014; 14: 54.
35. Fernandes M, Vira D, Medikonda R, Kumar N. Extensively and pan-drug resistant *Pseudomonas aeruginosa* keratitis: clinical features, risk factors, and outcome. *Graefes Arch Clin Exp Ophthalmol* 2016; 254: 315–322.
36. Gebremariam TT. Bacteriology and risk factors of bacterial keratitis in jimma, Southwest Ethiopia. *Ethiop Med J* 2015; 53: 191–197.
37. Gupta S, Mittal S, Nayak N, Satpathy G, Khokhar S, Agarwal T. *In vitro* antibiotic susceptibility of Pseu*domonas aeruginosa* corneal ulcer isolates. *Ocul Immunol Inflamm* 2015; 23: 252–255.
38. Hernandez-Camarena JC, Graue-Hernandez EO, Ortiz-Casas M et al. Trends in microbiological and antibiotic sensitivity patterns in infectious keratitis: 10-year experience in Mexico City. *Cornea* 2015; 34: 778–785.
39. Hsiao CH, Sun CC, Yeh LK et al. Shifting trends in bacterial keratitis in Taiwan: A 10-year review in a tertiary-care hospital. *Cornea* 2016; 35: 313–317.
40. Kaliamurthy J, Kalavathy CM, Parmar P, Nelson Jesudasan CA, Thomas PA. Spectrum of bacterial keratitis at a tertiary eye care centre in India. *Biomed Res Int* 2013; 2013: 181564.
41. Kowalski RP, Kowalski TA, Shanks RM, Romanowski EG, Karenchak LM, Mah FS. *In vitro* comparison of combination and monotherapy for the empiric and optimal coverage of bacterial keratitis based on incidence of infection. *Cornea* 2013; 32: 830–834.
42. Lim NC, Lim DK, Ray M. Polymicrobial versus monomicrobial keratitis: a retrospective comparative study. *Eye Contact Lens* 2013; 39: 348–354.
43. Oldenburg CE, Lalitha P, Srinivasan M et al. Emerging moxifloxacin resistance in *Pseudomonas aeruginosa* keratitis isolates in South India. *Ophthalmic Epidemiol* 2013; 20: 155–158.
44. Orlans HO, Hornby SJ, Bowler IC. In vitro antibiotic susceptibility patterns of bacterial keratitis isolates in Oxford, UK: a 10-year review. *Eye (Lond)* 2011; 25: 489–493.
45. Pandita A, Murphy C. Microbial keratitis in Waikato, New Zealand. *Clin Exp Ophthalmol* 2011; 39: 393–397.
46. Prokosch V, Gatzioufas Z, Thanos S, Stupp T. Microbiological findings and predisposing risk factors in corneal ulcers. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 369–374.
47. Ray KJ, Prajna L, Srinivasan M et al. Fluoroquinolone treatment and susceptibility of isolates from bacterial keratitis. *JAMA Ophthalmol* 2013; 131: 310–313.
48. Reinprayoon U, Sitthanon S, Kasetsuwan N, Chongthaleong A. Bacteriological findings and antimicrobial susceptibility pattern of isolated pathogens from visual threatening ocular infections. *J Med Assoc Thai* 2015; 98 Suppl 1: S70–76.
49. Sand D, She R, Shulman IA, Chen DS, Schur M, Hsu HY. Microbial keratitis in Los Angeles: the Doheny Eye Institute and the Los Angeles County Hospital experience. *Ophthalmology* 2015; 122: 918–924.
50. Chaurasia S, Ramappa M, Ashar J, Sharma S. Neonatal infectious keratitis. *Cornea* 2014; 33: 673–676.
51. Hong J, Chen J, Sun X et al. Paediatric bacterial keratitis cases in Shanghai: microbiological profile, antibiotic susceptibility and visual outcomes. *Eye (Lond)* 2012; 26: 1571–1578.
52. Lee YS, Tan HY, Yeh LK et al. Pediatric microbial keratitis in Taiwan: clinical and microbiological profiles, 1998-2002 versus 2008-2012. *Am J Ophthalmol* 2014; 157: 1090–1096.
53. Asbell PA, Sanfilippo CM, Pillar CM, DeCory HH, Sahm DF, Morris TW. Antibiotic resistance among ocular pathogens in the United States: Five-year results from the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) surveillance study. *JAMA Ophthalmol* 2015; 133: 1445–1454.
54. Haas W, Pillar CM, Torres M, Morris TW, Sahm DF. Monitoring antibiotic resistance in ocular microorganisms: results from the Antibiotic Resistance Monitoring in Ocular micRorganisms (ARMOR) 2009 surveillance study. *Am J Ophthalmol* 2011; 152: 567–574.e563.
55. Hajoui FZ, Daoudi N, Kabbaj H, Alaoui A, Seffar M. Bacteriological study of the strains isolated from ocular infections. *Ann Biol Clin (Paris)* 2012; 70: 689–694.
56. Jean SS, Coombs G, Ling T et al. Epidemiology and antimicrobial susceptibility profiles of pathogens causing urinary tract infections in the Asia-Pacific region: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2010-2013. *Int J Antimicrob Agents* 2016; 47: 328–334.
57. Qadeer A, Akhtar A, Ain QU et al. Antibiogram of medical intensive care unit at tertiary care hospital setting of Pakistan. *Cureus* 2016; 8: e809.
58. Rutter WC, Burgess DR, Burgess DS. Increasing incidence of multidrug resistance among cystic fibrosis respiratory bacterial isolates. *Microb Drug Resist* 2017; 23: 51–55.
59. Malhotra S, Mehta DK, Kumar P. Spectrum and antibiotic susceptibility pattern of bacterial isolates from conjunctival swabs. *Indian J Pathol Microbiol* 2005; 48: 538–541.
60. Maurer PP, Zbinden R, Kaufmann C, Thiel MA. Antibiotic susceptibilities of bacteria isolated from ophthalmic specimens between 1984 and 2005 in Zurich, Switzerland. *Klin Monbl Augenheilkd* 2007; 224: 240–243.
61. Smitha S, Lalitha P, Prajna VN, Srinivasan M. Susceptibility trends of *Pseudomonas* species from corneal ulcers. *Indian J Med Microbiol* 2005; 23: 168–171.
62. Ventola CL. The antibiotic resistance crisis: Part 1: causes and threats. *P T* 2015; 40: 277–283.
63. Yayan J, Ghebremedhin B, Rasche K. Antibiotic resistance of *Pseudomonas aeruginosa* in

pneumonia at a single university hospital center in Germany over a 10-year period. *PLoS One* 2015; 10: e0139836.
64. Bennett PM. Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *Br J Pharmacol* 2008; 153 Suppl 1: S347–357.
65. Stanisich VA. Identification and analysis of plasmids at the genetic level. *Method Microbiol* 1988; 21: 11–47.
66. Clewell DB, Weaver KE, Dunny GM, Coque TM, Francia MV, Hayes F. Extrachromosomal and mobile elements in Enterococci: Transmission, maintenance, and epidemiology. In: Gilmore MS, Clewell DB, Ike Y, Shankar N eds. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. Boston: Massachusetts Eye and Ear Infirmary, 2014.
67. Craig NL. Target site selection in transposition. *Annu Rev Biochem* 1997; 66: 437–474.
68. Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006; 4: 608–620.
69. Cury J, Jove T, Touchon M, Neron B, Rocha EP. Identification and analysis of integrons and cassette arrays in bacterial genomes. *Nucleic Acids Res* 2016; 44: 4539–4550.
70. Tato M, Coque TM, Baquero F, Canton R. Dispersal of carbapenemase blaVIM-1 gene associated with different Tn402 variants, mercury transposons, and conjugative plasmids in *Enterobacteriaceae* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2010; 54: 320–327.
71. Akingbade OA, Balogun S, Ojo DA et al. Plasmid profile analysis of multidrug resistant *Pseudomonas aeruginosa* isolated from wound infections in South West, Nigeria. *World Applied Sci J* 2012; doi: 10.5829/idosi.wasj.2012.20.06.667.
72. Olayinka AT, Olayinka BO, Onile BA. Antibiotic susceptibility and plasmid pattern of *Pseudomonas aeruginosa* from the surgical unit of a university teaching hospital in north central Nigeria. *Internat J Med Med Sci* 2009; 1: 5.
73. Mahmoud A, Zahran W, Hindawi G, Labib A, Galal R. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* in patients with nosocomial infections at a University hospital in Egypt, with special reference to typing methods. *J Virol Microbiol* 2013: 1–13.
74. Nikbin VS, Abdi-Ali A, Feizabadi MM, Gharavi S. Pulsed field gel electrophoresis & plasmid profile of *Pseudomonas aeruginosa* at two hospitals in Tehran, Iran. *Indian J Med Res* 2007; 126: 146–151.
75. Ehiaghe FA, Ehiaghe IJ, Agbonlahor DE et al. Plasmid profiling and curing analysis of fluoroquinolone multidrug resistant *Pseudomonas aeruginosa* in Benin city, Nigeria. *Open J Med Microbiol* 2013; 03: 201–205.
76. Thomas BT, Agu GC, Makanjuola SO, Davies A. Plasmid profiling and antibiotic resistance of extended spectrum beta lactamases producing *Pseudomonas aeruginosa* expressing Ampc beta-lactamase enzyme. *Am-Eurasian J Sci Res* 2015; 10: 9.
77. Koura BA, Zaghloul MHE, Mashad NE. Plasmid profile of multidrug resistant *Pseudomonas aeruginosa* strains in a burn unit. *Molec Diagnosis Vaccines* 2003; 1: 11.
78. Gibb AP, Tribuddharat C, Moore RA et al. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla(IMP) allele, bla(IMP-7). *Antimicrob Agents Chemother* 2002; 46: 255–258.
79. Kouda S, Ohara M, Onodera M et al. Increased prevalence and clonal dissemination of multidrug-resistant *Pseudomonas aeruginosa* with the blaIMP-1 gene cassette in Hiroshima. *J Antimicrob Chemother* 2009; 64: 46–51.
80. Pagani L, Colinon C, Migliavacca R et al. Nosocomial outbreak caused by multidrug-resistant *Pseudomonas aeruginosa* producing IMP-13 Metallo-beta-Lactamase. *J Clin Microbiol* 2005; 43: 3824–3828.
81. Tsakris A, Poulou A, Kristo I et al. Large dissemination of VIM-2-metallo-beta-lactamase-producing *Pseudomonas aeruginosa* strains causing health care-associated community-onset infections. *J Clin Microbiol* 2009; 47: 3524–3529.
82. Yousefi S, Nahaei M, Farajnia S et al. Class 1 integron and imipenem resistance in clinical isolates of *Pseudomonas aeruginosa*: Prevalence and antibiotic susceptibility. *Iran J Microbiol* 2010; 2: 115–121.
83. Xu Z, Li L, Shirtliff ME, Alam MJ, Yamasaki S, Shi L. Occurrence and characteristics of class 1 and 2 integrons in *Pseudomonas aeruginosa* isolates from patients in southern China. *J Clin Microbiol* 2009; 47: 230–234.
84. Fonseca EL, Vieira VV, Cipriano R, Vicente AC. Class 1 integrons in *Pseudomonas aeruginosa* isolates from clinical settings in Amazon region, Brazil. *FEMS Immunol Med Microbiol* 2005; 44: 303–309.
85. Keay L, Edwards K, Stapleton F. Referral pathways and management of contact lens-related microbial keratitis in Australia and New Zealand. *Clin Exp Ophthalmol* 2008; 36: 209–216.
86. Dart JK, Seal DV. Pathogenesis and therapy of *Pseudomonas aeruginosa* keratitis. *Eye (Lond)* 1988; 2 Suppl: S46–55.
87. Sanfilippo CM, Morrissey I, Janes R, Morris TW. Surveillance of the activity of aminoglycosides and fluoroquinolones against ophthalmic pathogens from Europe in 2010-2011. *Curr Eye Res* 2016; 41: 581–589.
88. Willcox MD. Management and treatment of contact lens-related *Pseudomonas* keratitis. *Clin Ophthalmol* 2012; 6: 919–924.
89. Livermore DM, Woodford N. The beta-lactamase threat in *Enterobacteriaceae, Pseudomonas* and *Acinetobacter*. *Trends Microbiol* 2006; 14: 413–420.
90. Wilke MS, Lovering AL, Strynadka NC. Beta-lactam antibiotic resistance: a current structural perspective. *Curr Opin Microbiol* 2005; 8: 525–533.
91. Dever LA, Dermody TS. Mechanisms of bacterial resistance to antibiotics. *Arch Intern Med* 1991; 151: 886–895.
92. Sanders CC. Inducible beta-lactamases and non-hydrolytic resistance mechanisms. *J Antimicrob Chemother* 1984; 13: 1–3.
93. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010; 54: 969–976.
94. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev* 2009; 22: 161–182.
95. Wolter DJ, Lister PD. Mechanisms of beta-lactam resistance among *Pseudomonas aeruginosa*. *Curr Pharm Des* 2013; 19: 209–222.
96. Papanicolaou GA, Medeiros AA, Jacoby GA. Novel plasmid-mediated beta-lactamase (MIR-1) conferring resistance to oxyimino- and alpha-methoxy beta-lactams in clinical isolates of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 1990; 34: 2200–2209.
97. Shahid M, Malik A, Sheeba. Multidrug-resistant *Pseudomonas aeruginosa* strains harbouring R-plasmids and AmpC beta-lactamases isolated from hospitalised burn patients in a tertiary care hospital of North India. *FEMS Microbiol Lett* 2003; 228: 181–186.
98. Zhu B, Zhang P, Huang Z et al. Study on drug resistance of *Pseudomonas aeruginosa* plasmid-mediated AmpC beta-lactamase. *Mol Med Rep* 2013; 7: 664–668.
99. Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci* 1980; 289: 321–331.
100. Jacoby GA, Matthew M. The distribution of beta-lactamase genes on plasmids found in *Pseudomonas*. *Plasmid* 1979; 2: 41–47.
101. Naas T, Philippon L, Poirel L, Ronco E, Nordmann P. An SHV-derived extended-spectrum β-lactamase in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1999; 43: 1281–1284.
102. Shaikh S, Fatima J, Shakil S, Rizvi SM, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci* 2015; 22: 90–101.
103. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991; 35: 147–151.
104. Ghamgosha M, Shahrekizahedani S, Kafilzadeh F, Bameri Z, Taheri RA, Farnoosh G. Metallo-beta-Lactamase VIM-1, SPM-1, and IMP-1 genes among clinical *Pseudomonas aeruginosa* species isolated in Zahedan, Iran. *Jundishapur J Microbiol* 2015; 8: e17489.
105. Lee K, Park AJ, Kim MY et al. Metallo-beta-lactamase-producing *Pseudomonas* spp. in Korea: high prevalence of isolates with VIM-2 type and emergence of isolates with IMP-1 type. *Yonsei Med J* 2009; 50: 335–339.
106. Queenan AM, Bush K. Carbapenemases: the versatile β-Lactamases. *Clin Microbiol Rev* 2007; 20: 440–458.
107. Bharathi MJ, Ramakrishnan R, Shivakumar C, Meenakshi R, Lionalraj D. Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. *Indian J Ophthalmol* 2010; 58: 497–507.
108. Jayahar Bharathi M, Ramakrishnan R, Ramesh S, Murugan N. Extended-spectrum beta-lactamase-mediated resistance among bacterial isolates recovered from ocular infections. *Ophthalmic Res* 2012; 47: 52–56.
109. Aggarwal R, Chaudhary U, Bala K. Detection of extended-spectrum beta-lactamase in *Pseudomonas aeruginosa*. *Indian J Pathol Microbiol* 2008; 51: 222–224.
110. Rameshkumar G, Ramakrishnan R, Shivkumar C et al. Prevalence and antibacterial resistance patterns of extended-spectrum beta-lactamase producing Gram-negative bacteria isolated from ocular infections. *Indian J Ophthalmol* 2016; 64: 303–311.
111. Murugan N, Malathi J, Umashankar V, Madhavan HN. Resistome and pathogenomics of multidrug resistant (MDR) Pseudomonas aeruginosa VRFPA03, VRFPA05 recovered from alkaline chemical keratitis and post-operative endophthalmitis patient. *Gene* 2016; 578: 105–111.

112. Griffith LJ. Development of resistance to kanamycin. *Ann N Y Acad Sci* 1966; 132: 796–799.
113. Edson RS, Terrell CL. The aminoglycosides. *Mayo Clin Proc* 1999; 74: 519–528.
114. Poole K. Aminoglycoside Resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2005; 49: 479–487.
115. Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* 1993; 57: 138–163.
116. Benveniste R, Davies J. Mechanisms of antibiotic resistance in bacteria. *Annu Rev Biochem* 1973; 42: 471–506.
117. Courvalin P, Carlier C. Resistance towards aminoglycoside-aminocyclitol antibiotics in bacteria. *J Antimicrob Chemother* 1981; 8 Suppl A: 57–69.
118. Araujo BF, Ferreira ML, Campos PA et al. Clinical and molecular epidemiology of multidrug-resistant *P. aeruginosa* carrying aac (6′)-Ib-cr, qnrS1 and blaSPM genes in Brazil. *PLoS One* 2016; 11: e0155914.
119. Dubois V, Arpin C, Noury P, Quentin C. Clinical strain of *Pseudomonas aeruginosa* carrying a bla(TEM-21) gene located on a chromosomal interrupted TnA type transposon. *Antimicrob Agents Chemother* 2002; 46: 3624–3626.
120. Schwocho LR, Schaffner CP, Miller GH, Hare RS, Shaw KJ. Cloning and characterization of a 3-N-aminoglycoside acetyltransferase gene, aac(3)-Ib, from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1995; 39: 1790–1796.
121. Kazama H, Kizu K, Iwasaki M, Hamashima H, Sasatsu M, Arai T. Isolation and structure of a new integron that includes a streptomycin resistance gene from the R plasmid of *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* 1995; 134: 137–141.
122. Girlich D, Naas T, Leelaporn A, Poirel L, Fennewald M, Nordmann P. Nosocomial spread of the integron-located veb-1-like cassette encoding an extended-pectrum beta-lactamase in *Pseudomonas aeruginosa* in Thailand. *Clin Infect Dis* 2002; 34: 603–611.
123. Miyoshi-Akiyama T, Kuwahara T, Tada T, Kitao T, Kirikae T. Complete genome sequence of highly multidrug-resistant *Pseudomonas aeruginosa* NCGM2.S1, a representative strain of a cluster endemic to Japan. *J Bacteriol* 2011; 193: 7010.
124. Sajid Al-Jubori S, Ameen Al-Jabiri H, M.S. Al-Kadmy I. Molecular detection of aminoglycoside resistance mediated by efflux pump and modifying enzymes in *Pseudomonas aeruginosa* isolated from Iraqi hospitals. In. *Int'l Conf on Medical Genetics, Cellular & Molecular Biology*, Pharmaceutical & Food Sciences. Istanbul, 2015.
125. Hamed SM, Aboshanab KMA, Elkhatib WF, Ashour MS. Aminoglycoside resistance patterns of certain gram negative uropathogens recovered from hospitalized Egyptian patients. *Br Microbiol Res Jl* 2013; 3: 14.
126. Sekiguchi J, Asagi T, Miyoshi-Akiyama T et al. Multidrug-resistant *Pseudomonas aeruginosa* strain that caused an outbreak in a neurosurgery ward and its aac(6′)-Iae gene cassette encoding a novel aminoglycoside acetyltransferase. *Antimicrob Agents Chemother* 2005; 49: 3734–3742.
127. Bi D, Xie Y, Tai C et al. A site-specific integrative plasmid found in *Pseudomonas aeruginosa* clinical isolate HS87 along with a plasmid carrying an aminoglycoside-resistant gene. *PLoS One* 2016; 11: e0148367.
128. Kobayashi K, Hayashi I, Kouda S et al. Identification and characterization of a novel aac(6′)-Iag associated with the blaIMP-1-integron in a multidrug-resistant *Pseudomonas aeruginosa*. *PLoS One* 2013; 8: e70557.
129. Blondeau JM. Fluoroquinolones: mechanism of action, classification, and development of resistance. *Surv Ophthalmol* 2004; 49 Suppl 2: S73–78.
130. Smith A, Pennefather PM, Kaye SB, Hart CA. Fluoroquinolones: place in ocular therapy. *Drugs* 2001; 61: 747–761.
131. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005; 41 Suppl 2: S120–126.
132. Lee JK, Lee YS, Park YK, Kim BS. Alterations in the GyrA and GyrB subunits of topoisomerase II and the ParC and ParE subunits of topoisomerase IV in ciprofloxacin-resistant clinical isolates of *Pseudomonas aeruginosa*. *Int J Antimicrob Agents* 2005; 25: 290–295.
133. Martinez-Martinez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; 351: 797–799.
134. Cattoir V, Nordmann P. Plasmid-mediated quinolone resistance in gram-negative bacterial species: an update. *Curr Med Chem* 2009; 16: 1028–1046.
135. Cayci YT, Coban AY, Gunaydin M. Investigation of plasmid-mediated quinolone resistance in *Pseudomonas aeruginosa* clinical isolates. *Indian J Med Microbiol* 2014; 32: 285–289.
136. Tran QT, Nawaz MS, Deck J, Nguyen KT, Cerniglia CE. Plasmid-mediated quinolone resistance in *Pseudomonas putida* isolates from imported shrimp. *Applied Environmental Microbiol* 2011; 77: 1885–1887.
137. Aggarwal RK, Dawar C, Das S, Sharma S. Draft genome sequences of two drug-resistant isolates of *Pseudomonas aeruginosa* obtained from keratitis patients in India. *Genome Announc* 2015; 3: e01404–14.
138. Kaye S, Tuft S, Neal T et al. Bacterial susceptibility to topical antimicrobials and clinical outcome in bacterial keratitis. *Invest Ophthalmol Vis Sci* 2010; 51: 362–368.
139. Wilhelmus KR, Abshire RL, Schlech BA. Influence of fluoroquinolone susceptibility on the therapeutic response of fluoroquinolone-treated bacterial keratitis. *Arch Ophthalmol* 2003; 121: 1229–1233.
140. Kucken D, Feucht H, Kaulfers P. Association of qacE and qacEDelta1 with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS Microbiol Lett* 2000; 183: 95–98.