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Review Article

Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa* and a Multi-Pronged Approach to Combat its Infection in Veterinary Science and Public Health: A Review

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Abstract

Pseudomonas aeruginosa is one of the most important nosocomial pathogens associated with a variety of medical and veterinary infections and therefore, it presents a major public health threat. Different classes of antibiotics are being used to treat its infections which are increasing selective pressure to multi-drug resistance development. Resistance to antibiotics in *P. aeruginosa* is due to many of the common and unique mechanisms which include: reducing membrane permeability, modification or inactivation of antibiotics, alteration of enzymes, modification of target sites and over-expression of efflux systems. Over or under expression of the genes of porin channels and components of efflux systems play a major role in the resistance mechanisms of *P. aeruginosa*. To overcome the problem of the emergence of antibiotic resistance, many new strategies are being employed to control infections caused by *P. aeruginosa*. These include the use of herbs/medicinal plants and phage therapy. With the advent of modern technology, the molecular mechanisms of these alternative therapies are being elucidated and may be used in future to treat *P. aeruginosa* infections in humans and veterinary clinics. This review thus highlights the mechanisms of antibiotic resistance of *P. aeruginosa* against the commonly used antimicrobials and also some alternative strategies to control *P. aeruginosa* infection.

Keywords: *Pseudomonas*; Antibiotic resistance; Efflux pump; Disinfectant; Porin

Introduction

Antimicrobial therapy has remained beneficial in treating infectious diseases caused by bacteria. During exposure to antibiotics, pathogens have developed resistance and have become a challenge for healthcare professionals. Among Gram-negative bacteria, *Escherichia coli*, *Acinetobacter baumanni* and *Klebsiella pneumoniae* have become resistant to many antibiotics but the most important pathogen which has shown resistance to all classes of antibiotics is *Pseudomonas aeruginosa (P. aeruginosa)* (Breidenstein *et al.* 2011). The organism is a gram-negative; motile bacterium that lacks fermentative properties (which differentiates it from *Enterobacteriaceae*). It can grow in harsh conditions with low levels of nutrients, bearing the temperature range from 4ºC to 44ºC. It is an opportunistic microorganism, which is associated with a variety of infections including cystitis, pneumonitis, gastritis, otitis and keratitis in immunocompromised individuals. It is not present in normal human microflora, but it is one of the most notorious organisms responsible for nosocomial infections. It colonizes moist places in hospitals including benchtop surfaces, surgical instruments, urinary catheters and intravenous catheters. *P. aeruginosa* can survive in disinfectants, making it unique as compared to other pathogens (Chakraborty *et al.* 2016). Chances of its infection increase with the increase in time of hospitalization (Ferstl *et al.* 2016).

A variety of factors play roles in the pathogenesis of *P. aeruginosa* including virulence factors. Factors associated with its surface are lipopolysaccharide, rhamnolipids, flagella, mucus and fimbriae, while enzymatic virulence factors include alkaline protease, elastase, hemolysin, neuraminidase, gelatinase, and phospholipases (van 't Wout *et al.* 2015). These virulence factors help in evading the human immune system and establishing the infection (Khalil *et al.* 2015). Mechanisms of its resistance are intrinsic as well as extrinsic, depending upon the nature of

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genes involved. Intrinsic mechanisms involve genes from its chromosome while acquired resistance can be obtained from plasmids or bacteriophages, by horizontal gene transfer. Infections of *P. aeruginosa* are difficult to control due to its wide variety of virulence factors and resistance mechanisms. In this review, we highlight the resistance mechanisms against different antibiotics. Understanding of these mechanisms will help in the judicious use of antibiotics and the study of new ways to control this pathogen.

Mechanisms of antibiotic resistance

All antimicrobials must penetrate the cell wall of a bacterium to be effective. *Pseudomonas aeruginosa* is resistant to a variety of antibiotics, primarily because it offers limited entry to different antimicrobials. It has a polysaccharide barrier (alginate) around it, which is anionic and can effectively bind with the cationic antimicrobials *e.g*. aminoglycosides and restricts their entry into the cell (Germoni *et al.* 2016). Moreover, the outer membrane of *P. aeruginosa* also acts as a major barrier in the passage of different molecules which are larger and hydrophilic in nature. Hydrophilic antimicrobials of small molecular size can easily cross the outer membrane of the bacteria by passage through porins (Song *et al.* 2015).

Decreasing permeability

Outer membranes of Gram-negative bacteria contain heavy outer membrane proteins in them. These proteins are porin channel *i.e*., oprJ, oprN, oprM, oprN which help in the transport of molecules but resist the antimicrobials physically or in combination with active efflux pumps. *Pseudomonas aeruginosa* provides less membrane permeability to antimicrobials as compared to the members of *Enterobacteriaceae* due to the high ratio of porins. Porin proteins and efflux pumps work side by side to increase their resistance. oprF is a major type of porin produced by all strains of the *P. aeruginosa*. OprD is another important porin involved principally in the uptake of lysine (and other positively charged amino acids). If this porin is lost, it results in the resistance to the meropenem (Fluit *et al.* 2019) and carbapenem (Richardot *et al.* 2015). Another porin is OprH which prevents the binding of antimicrobials to the lipopolysaccharides (LPS) of bacteria thus preventing the uptake of those compounds (Qadi *et al.* 2016).

Efflux system (multidrug)

Different families of efflux system are the protein pumps involved in resistance for the transport of substances across the bacterial membrane and cell wall. One of the families is resistance-nodulation-division (RND) which characterizes both impermeability resistance and adaptive resistance to multiple antibiotics in *P. aeruginosa*. It is primarily composed of three major components that include 1) an inner membrane pump (the RND component), 2) An outer membrane channel-forming porin and 3) a periplasmic linker protein which joins the two components together (Fig. 1A, B, C, D). While writing the name of the efflux pump, periplasmic linker and the inner membrane pump come first followed by the type of porin (Daury *et al.* 2016). Different types of efflux systems which are reported for the *P. aeruginosa* are mexAB-oprM (mexA = periplasmic linker, mexB = membrane pump, oprM = porin), mexCD-oprJ, mexEF-oprN, mexXY-oprM and mexXY-oprN. The most commonly present efflux pump is the mexAB-oprM while mexXY-oprN is less commonly found. The activity of mexXY-oprN is dependent on the presence or absence of porin oprD, as its range of resistance is increased with the absence of oprD porin (Kao *et al.* 2016). Common types of efflux pumps are mentioned in Table 1.

MexAB-OprM efflux system participates in both intrinsic as well as acquired resistance in *P. aeruginosa*, but MexEF-OprN and MexCD-OprJ contribute only to the acquired resistance. Role of these efflux pumps was confirmed by the knock-out gene mutations. At the start, oprK was considered to be the porin channel of MexAB efflux pump and was referred as MexAB-oprK pump but later on, oprM was found to be the porin of MexAB efflux pump and named as MexAB-oprM efflux pump (Baranova 2016). Moreover, the efflux system also plays an important role in inducing resistance against meropenem (Rostami *et al.* 2018).

Modification and inactivation of antibiotics

The AmpC gene is possessed by all *Pseudomonas* strains. This gene primarily produces resistance to beta-lactam antibiotics. AmpR is a regulatory gene which is responsible for the overproduction of beta-lactamase by *P. aeruginosa* strains. Beta-lactamase is present in the periplasmic space of *P. aeruginosa*. Beta-lactamases, as well as integron and plasmid-encoded extended-spectrum beta-lactamases (ESBLs), are responsible for contributing resistance against cephalosporin and penicillin. Beta-lactamase inhibitors interfere with the enzyme by plasmid but not by AmpC gene (Buberg *et al.* 2020).

In the case of *P. aeruginosa*, this mechanism has been observed against quinolones and penicillins. Quinolones bind to DNA gyrase enzyme. A *gyrA* mutation results in the change of the DNA gyrase enzyme, thus making quinolones ineffective (Park *et al.* 2020). *Pseudomonas* strains associated with cystic fibrosis exhibit a frequent change in the penicillin-binding proteins (PBP's) resulting in the resistance against the penicillin group. mRNA expression levels of oprM and ampC genes led to 21.7 and 25% resistance respectively. Point mutation of AmpR at Asp135-Asn and Als194-Ser deregulated the ampC induction and led to 21.7% resistance in *Pseudomonas* isolates (Du *et al.* 2010).

Table 1: Common types of multi-drug resistance efflux pumps with their resistant and susceptible antibiotics

Periplasmic Linker	Inner membrane Pump	Porin	Resistance to Antimicrobials	Antimicrobials not affected
MexA	MexB	OprM	Quinolone, Macrolide, Tetracycline, Chloramphenicol, anti-pseudomonal Penicillins, anti-pseudomonal Cephalosporins, Disinfectants	Aminoglycoside, Imipenem
MexC	MexD	OprJ	Quinolone, Macrolide, Tetracycline, Chloramphenicol,	Carbenicillin, Sulbenicillin, Ceftazidime
MexE	MexF	OprN	Fluoroquinolone, Chloramphenicol, Trimethoprim, Carbapenems	Ticarcillin, Cefepime, Aztreonam, Aminoglycoside
MexX	MexY	OprM	Quinolone, Macrolide, Tetracycline, Chloramphenicol, β-lactams, Aminoglycosides	Carbenicillin, Sulbenicillin, Cefsulodin, Ceftazidime

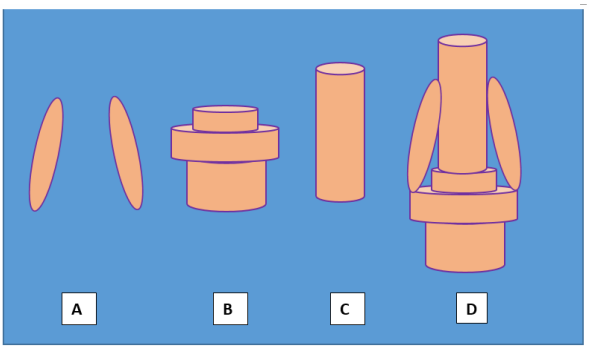

Fig. 1: A; Periplasmic linker protein, **B**; Inner membrane pump, **C**; Channel porin, **D**; Complete Efflux pump

Induction of biofilm

Pseudomonas aeruginosa colonization appears as the aggregate of the cells, surrounded by the protective coating of polysaccharide, alginate, proteins and extracellular DNA. These biofilms are becoming a major source of resistance against antibiotics and disinfectants (Hameed *et al.* 2017). *Pseudomonas aeruginosa* effectively forms biofilms when exposed to the sub-optimal concentrations of the different antibiotics like tobramycin, tetracycline and ciprofloxacin. Tobramycin is also responsible for the induction of swimming and swarming in *P. aeruginosa* (Linares *et al.* 2006). Biofilm resists by interacting with different mechanisms simultaneously. Biofilm matrix allows limited penetration of antibiotics towards bacterial cells. Bacterial cells produce a limited number of inactive cells in the biofilm matrix that do not grow or die even in the presence of antibiotics, called persister cells. These cells have the special ability to tolerate multiple antibiotics after their diffusion through the matrix (Dawson *et al.* 2011). Extracellular DNA having negative charge binds with the cationic antimicrobial peptides including polymyxin to restrict their entry into the cell. Moreover, exposure of pseudomonal biofilms to the imipenem resulted in enhanced expression of genes responsible for the biosynthesis of alginate, which results in the production of stronger biofilm formation (Olivares *et al.* 2020).

Resistance against beta-lactam ring containing antibiotics

P. aeruginosa is resistant against many compounds which may be structurally related or unrelated. Intrinsically, it is resistant to many antimicrobial compounds due to β lactamases against penicillin G, aminopenicillins and cephalosporins. Beta-lactam antibiotics lead to induction of the AmpC gene which is chromosomally encoded but horizontal gene transfer increases resistance as well. The incidence of ESβL resistant strains between *P. aeruginosa* isolates was found 26.86 and 25.14% in nosocomial and community sources, respectively (Sahu *et al.* 2012). Moreover, 25.3 and 28.7% *P. aeruginosa* isolates were resistant to meropenem and imipenem (Hu *et al.* 2017). The resistance is due to outer membrane permeability and efflux pumps (transporters) that actively push beta-lactam antibiotics out of the cell and also got the ability to contain some of the enzymes which can inactivate the antimicrobial drugs *e.g.*, penicillinases and cephalosporinases (Xu *et al.* 2020). Carbapenems are considered as last resort antibiotics for the treatment of *P. aeruginosa* along with colistin (Manohar *et al.* 2018).

Induction of AmpC gene and the extent of hydrolysis of antibiotics decide the fate of beta-lactams against *P. aeruginosa*. Amoxicillin, 1st and 2nd generation cephalosporins are relatively ineffective against *P. aeruginosa* infections due to their easy hydrolysis and strong induction of AmpC. An important group of beta-lactam antibiotics that are used to treat the infection of *P. aeruginosa* include ureidopenicillins (azlocillin), carboxypenicillins (ticarcillin and carbenicillin), 3rd & 4th generation cephalosporins (ceftazidime and cefoperazone), aztreonams and carbapenems (meropenem and imipenem) (Bagge *et al.* 2002). This is leading to the appearance of new β-lactamases by natural evolution.

Aminoglycosides

Aminoglycosides are bactericidal and they do this by binding to the 30S subunit of bacterial ribosomes, misreading of the codons and thus causing the death of microorganisms (Dunkle *et al.* 2014). *Pseudomonas aeruginosa* is naturally resistant to some of the aminoglycosides like kanamycin because it can easily phosphorylate it (Kondo and Hotta 1999). The uptake of the aminoglycosides is a complex process which involves binding with LPS and permeability offered by the outer membrane of Gram-negative bacteria, then crossing of substances across the plasma membrane due to action potential and ultimately binding with ribosomes and disrupting the polypeptide synthesis in bacteria

(Krahn *et al.* 2012). *Pseudomonas aeruginosa* has developed resistance which causes methylation of nucleotides due to horizontal gene transfer.

Strains of *P. aeruginosa* isolated from the clinical and laboratory studies have shown both intrinsic and adaptive type of resistance against aminoglycosides. It is well documented that aminoglycosides can also be antagonized by certain ions of divalent nature *i.e.*, Ca and Mg particularly in the case of *P. aeruginosa* (Morita *et al.* 2012). A recent analysis at the transcriptomic level has shown that aminoglycosides can affect the variety of the genes of intrinsic and adaptive nature. *Pseudomonas aeruginosa*, when exposed to the tobramycin for quite a prolonged interval of time at a dose less than MIC can significantly enhance the expression of MexXY (efflux pump) genes. Heat shock genes (*i.e.*, groES, asrA, htpG and ibpA) are also overexpressed when *P. aeruginosa* is exposed to tobramycin at a sub-lethal concentration; among them, asrA is particularly important regarding resistance to aminoglycosides (Basta *et al.* 2020). Expression of chromosomal genes mexZ, rplY, PA5471, nuoG & galU, and MexXY–OprM efflux system increase aminoglycoside resistance in *P. aeruginosa* (Islam *et al.* 2009).

Resistance against tetracycline

Tetracycline group is primarily bacteriostatic in action and includes a variety of antimicrobial compounds. An energy-driven process is required to enter the tetracyclines inside the cell, where they bind to the 30S ribosomal subunit and interfere with the binding of aminoacyl-tRNA to the A site (acceptor) in the ribosomal-RNA complex. Intrinsically tetracyclines are ineffective against Pseudomonal infections because of the presence of the MexAB/mexXY multidrug efflux type of pumps (Konai and Haldar 2020).

Resistance against macrolides

Macrolides are a group of antimicrobials that also tend to interfere with the synthesis of proteins in microorganisms and they do this by attaching to the 50S subunit of ribosomes. These are commonly used to treat the pulmonary infections associated with *P. aeruginosa* (Laserna *et al.* 2014; Solleti *et al.* 2015). They also tend to induce type III secretion system of the bacteria. Low concentration (2 μg/mL) of macrolides enhances the production of some mutant strains *e.g. nfxB* mutants, which then effectively produce the efflux pumps *i.e.*, MexCD-OprJ. Chromosomally encoded expression of MexCD-OprJ and MexXY/MexAB-OprM efflux pumps interferes with the natural resistance of *P. aeruginosa* against the macrolide (Mulet *et al.* 2011).

Resistance against chloramphenicol

Chloramphenicol is primarily bacteriostatic that interferes with the multiplication of the microorganisms by binding to the 50S subunit of the bacterial ribosomes and they inhibit the peptidaltransferase enzyme. *Pseudomonas* is resistant against chloramphenicol intrinsically, mainly due to the presence of an efflux system of MexAB-oprM type (Fernández *et al.* 2012). Moreover, the sub-optimal doses of the drug lead to the induction of MexXY efflux system.

Resistance against fluoroquinolones

MexXY-OprM and MexAB-OprM contribute to quinolone resistance in wild-type *Pseudomonal* spp. (Morita *et al.* 2001). Efflux pumps have been found that are responsible for the resistance development in case of *Pseudomonas i.e.*, MexVW-OprM and MexHI-OpmD. MexEF-OprN and MexCD-OprJ efflux pumps are linked to quinolone resistance while MexCD-OprJ is related to multidrug resistance (Terzi *et al.* 2014).

Resistance against other biocides

Triclosan, chlorhexidine and benzalkonium can become contaminated with the *Pseudomonas*, therefore, making the disinfectant less efficient (Shepherd *et al.* 2018). Resistance against triclosan is primarily due to an efflux pump *i.e.*, MexAB-OprM (Mima *et al.* 2007; Zhu *et al.* 2010). The sub-optimal concentration of the chlorhexidine and benzalkonium lead to the resistance against *Pseudomonas* primarily by MexCD-OprJ efflux pump. *Pseudomonas aeruginosa* strains showing resistance against the benzalkonium also tend to become resistant against the quinolones due to mutations in the gyrA, mexCD-oprJ and mexAB-oprM (Mc Cay *et al.* 2010). Resistance mechanisms of *P. aeruginosa* have been summarized in Table 2.

Implications of anti-microbial resistance in veterinary and public health and employing alternative intervention strategies

In humans, *P. aeruginosa* is known to cause a myriad of clinical diseases such as sepsis, pneumonia, skin infections and cystic fibrosis (Jeong *et al.* 2014). So, these infections contribute significantly towards the higher morbidity, mortality and treatment cost. According to China antimicrobial surveillance network (CHINET), *P. aeruginosa* is one of the four Gram-negative bacteria to be isolated from each clinical specimen (Hu *et al.* 2017). In animals, *P. aeruginosa* is known to cause many diseases, including, chronic pyoderma, Urinary tract infections (UTIs), wound infections, otitis externa, bovine mastitis and feline septicemia (Ahmad 2001; Mekić *et al.* 2011; Maniam *et al.* 2019). *P. aeruginosa* isolated from the cats and dogs in Japan have shown 4.1, 12.3, 17.8, 20.5, 31.5 and 34.2% resistance against gentamicin, aztreonam, cefotaxime, ciprofloxacin, enrofloxacin and orbifloxacin. Ear isolates of *P. aeruginosa* have shown resistance to orbifloxacin, enrofloxacin and ciprofloxacin. Moreover, bacteria from

Table 2: Resistance mechanisms of *P. aeruginosa* against commonly used antimicrobials

Antimicrobial	Mechanism	Reference
β- lactams	Induction of AmpC β- lactamase	(Sahu *et al*. 2012)
Aminoglycosides	Expression of Heat shock genes; Alteration in MexXY–OprM	(Islam *et al.* 2009; Basta *et al*. 2020)
	Biofilm development	(Linares *et al*. 2006)
	Induction of swimming and swarming	(Linares *et al*. 2006)
Carbapenems	Development of thicker biofilm	(Olivares *et al.* 2020)
Chloramphenicol	Induction of Mex EF-OprN complex	(Fernández *et al*. 2012)
quinolones	Over-expressed MexEF-OprN and MexCD-OprJ	(Terzi *et al*. 2014)
Tetracycline	Over-expressed mexA-mexB-oprM	(Konai and Haldar 2020)
MDR	MexCD-OprJand MexAB-OprM	(Terzi *et al*. 2014)

urine were more resistant to many antimicrobial drugs than skin isolates (Harada *et al.* 2012). Similar findings have been demonstrated in a study that showed that *P. aeruginosa* isolated from canine otitis externa to be 47, 67 and 75% resistant against enrofloxacin, marbofloxacin and ciprofloxacin (Wildermuth *et al.* 2007). Similar trends of antimicrobial resistance have been shown in the *P. aeruginosa* isolates from mouth, skin, urogenital tract and ears (Werckenthin *et al.* 2007). In the recent past, it has been demonstrated that *P. aeruginosa* can cross different species barriers thereby indicating its potential as a zoonotic pathogen (Fernandes *et al.* 2018).

So, because of the presence of multidrug resistance in *P. aeruginosa*, there is a need for appropriate drug therapy to overcome this issue, because inappropriate antimicrobial therapy at initial disease stages can lead to enhanced morbidity and mortality. So, as done in the past, combinations of antibiotics (although controversial) may be employed to deal with the problem of multidrug resistance (Guan *et al.* 2016). Although colistin and amikacin exhibit anti-pseudomonal activity, but their toxicity deters their frequent use in clinics. So, in this aspect, new antibiotics that are known to possess activity against the multi-drug resistance (MDR) *P. aeruginosa*, they may be deployed prudently in clinics to curb the infection by MDR *P. aeruginosa*. Tazobactam/ Ceftolozane is a very effective antibacterial drug combination that is recommended to treat the intra-abdominal infections as well as UTIs. *In vitro* studies have demonstrated that the development of antimicrobial resistance against this combination is slower as compared to the other antibiotics (Tato *et al.* 2015). Moreover, a new combination of avibactam (non beta-lactam) with the 3rd generation cephalosporin (Ceftazidime) has also been approved for the treatment of UTIs and intra-abdominal infections (along with metronidazole) caused by *P. aeruginosa* (Sader *et al.* 2017; Xipell *et al*. 2017). Another study has also demonstrated that after colistin, the combination of Tazobactam/Ceftolozane and avibactam/Ceftazidime was found to be most effective against *P. aeruginosa* infections where 97.5 and 96.9% of *P. aeruginosa* population was found to be susceptible to both combinations, respectively (Sader *et al.* 2018). So, there is still much room to discover some novel antimicrobial combinations which may prove good antimicrobial activity both *in vitro* and under clinics against *P. aeruginosa*.

Alternatively, another approach to combat infections caused by *P. aeruginosa* may be by using various plant/herb based compounds, and this approach is much safer than using antimicrobials. Medicinal plants or herbs have a variety of compounds such as long-chained unsaturated aldehydes, peptides, essential oils, and phenolic compounds, which are known to possess the antimicrobial activity (Hameed and Ahmed 2014; Astal *et al.* 2005). It has been shown that ginseng supplementation results in the reduced *P. aeruginosa* and mast cells number in the lungs of rats, thereby significantly reducing the lung pathology (Nguyen and Nguyen 2019). Leave extract of *Azadirachta indica* (neem) are also found to inhibit the biofilms formation in *P. aeruginosa* (Harjai *et al.* 2013). One more study demonstrated that flavonoids extracted from *Moringa oleifera* possess the anti-biofilm activities of *P. aeruginosa* (Onsare and Arora 2015). Another study has found that Zingiber officinale (ginger), Glycyrrhiza glabra (liquorice) and *Mentha piperita* (mint), possess antipseudomonal activity against many MDR strains (Chakotiya *et al.* 2016). Although the exact mechanism by which these herbs/nutraceuticals impart antimicrobial effects largely remains to be elucidated, however, their phytochemicals play important role in the molecular mechanisms involved in their antimicrobial effects. *In vivo* mice model studies have shown that oral supplementation of garlic extract is useful in preventing the infections caused by *P. aeruginosa*. After oral administration, garlic was found to be effective in reducing the renal bacterial count. *In vitro* studies demonstrated the reduction in the signals responsible for quorum sensing and thereby reducing the release of various virulence factors (Harjai *et al.* 2010). A more recent study also demonstrated that purified flavonoids fraction derived from *Cassia alata* L. (*Ca. alata*), was found to be associated with the reduced release of virulence factors, reduction in quorum sensing signals and reduced biofilm formation by *P. aeruginosa* (Rekha *et al.* 2017). The latest study has demonstrated that quercetin (natural flavonoid compound) has been found to specifically inhibit the quorum sensing

and thus biofilm formation and virulence factors production by *P. aeruginosa* (Ouyang *et al.* 2016).

Recently phage therapy presented an alternative solution to multidrug-resistant bacteria and it offers several advantages over antibiotics. A single dose of phage can eradicate potentially infectious bacteria. However, till now, phages are reported to be genera and species (even strain) specific (Nguyen *et al.* 2012). Siphoviridae phages are known to be important in controlling *P. aeruginosa* infections(Yamaguchi *et al.* 2014). In a recent study, JHP phage has shown its antibacterial activity against a myriad of *P. aeruginosa* strains (Khawaja *et al.* 2016).

Conclusion

Resistance mechanisms are constantly evolving by natural evolution or horizontal gene transfer. There is a grave challenge to tackle infections these days as the antibiotic resistance keeps on increasing from multidrug to pan-drug resistance. These resistant strains can be treated by aggressive approach, avoiding the suboptimal doses. Infections should be treated with broad-spectrum antibiotics followed by narrowing down to the specific antibiotic till the sensitivity result comes. Antibiotic therapy can be used with supporting agents as beta-lactamase inhibitors *e.g*. Clavulanate or biofilm dissolving substances *e.g*., alginate lyases. There is also need to focus on the post-antibiotic era; screening drugs for efflux pump inhibitors, searching new types of beta-lactamase inhibitors, exploring cationic membrane permeabilizers, manipulating medicinal plants/herbs, enhancing quorum quenching to inhibit communication and biofilm formation and phage therapy to treat infections. Emphasis should also be done to improve the specific or general immune response of patients infected by *P aeruginosa* along with other non-fermentative bacterial infections.

Author Contributions

BEM and MAA conceived the idea, BEM acquired the data, BEM and MAA wrote the manuscript with the inputs of MKB and HB. MKB and HB worked on drafting and revising of the article critically.

Conflict of Interest

We declare that we do not have any conflict of interest.

Data Availability

Not applicable.

Ethics Approval

Not applicable.

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