REVIEW

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Novel therapies of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. infections: the state of the art

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Abstract

Gram-negative non-fermenting bacilli, particularly *Pseudomonas aeruginosa* and *Acinetobacter* spp., are important opportunistic pathogens in hosppitalized patients, contributing to their morbidity and mortality. Recently, a rapid increase in frequency of multidrug-resistant clinical strains is being recorded, making the available therapeutic options very limited. Apart from the development of novel classes of antimicrobials, there is renewed interest in the use of old agents or new combinations of available drugs. Numerous *in vitro* investigations have been reported on the efficacy of different antimicrobials; however, they should be evaluated in experimental infection models and clinical trials. Novel approaches are being investigated, such as inhibition of virulence factor expression by pathogens or inhibition of their metabolic pathways. The use of bacteriophages, particularly those genetically modified, remains an alternative option in the therapy of infections caused by multidrug-resistant strains. Several vaccines against *P. aeruginosa* are under development. Apart from therapy with antimicrobial agents, eradication of outbreaks comprises implementation of strict infection control measures and prudent use of antimicrobials.

Key words: *Pseudomonas*, *Acinetobacter*, vaccines, antimicrobial therapy.

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CLINICAL IMPORTANCE OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER* SPP.

The Gram-negative non-fermenting bacilli *Pseudomonas aeruginosa* and *Acinetobacter* spp. are important opportunistic pathogens. Frequency of infections caused by them is increasing and multidrug-resistant (MDR) strains, resistant to almost all available antimicrobials, are emerging in hospitalized patients. Therefore, as therapeutic options become limited, the search for novel agents becomes a priority.

Strains of *P. aeruginosa* and *Acinetobacter* spp. cause disease in hospitalized patients, predominantly pneumonia, bacteremia, meningitis, urinary tract infections, as well as skin and soft-tissue infections [25, 41, 46]. They are opportunistic pathogens, particularly dangerous to intensive care unit (ICU) patients due to the severity of their underlying disorders, older age, steroid therapy, and the administration of immunosuppressive drugs [4, 25]. Furthermore, *P. aeruginosa* often causes infections in patients with cystic fibrosis and bronchiectases. *P. aeruginosa* and *Acinetobacter* spp. are important etiological agents of infections in neutropenic and in ICU patients, particularly those with ventilator-associated pneumonia (VAP) [40, 55]. The mortality rate is high [25, 41]. *P. aeruginosa* bacteremia in ICU patients is linked to a clinically significant crude mortality of 15–78%, while attributable mortality ranges from 34 to 48% [4, 25]. Identification of risk factors for MDR pathogens causing VAP is therefore very important so that appropriate empiric therapy can be used, as this will greatly improve patient outcomes [55].

EMERGENCE OF MDR STRAINS

P. aeruginosa and *Acinetobacter* spp. are naturally resistant to a number of antimicrobials (Table 1). Furthermore, they easily acquire resistance to antibacterial

Table 1. Natural resistance of *P. aeruginosa* and *A. baumannii* to antibiotics

Bacteria	Natural resistance
P. aeruginosa	ampicillin amoxicillin amoxicillin/clavulanate first-generation cephalosporins second-generation cephalosporins cefotaxime ceftriaxone nalidixic acid trimethoprim
A. baumannii	ampicillin amoxicillin first-generation cephalosporins

agents by mutational changes or acquisition of genetic material. Resistance of both *P. aeruginosa* and *Acinetobacter* spp. to commonly used therapeutic agents has increased in recent years [22]. MDR can be defined as resistance to at least three classes of the antibiotics used in the treatment of these infections: third-generation cephalosporins, fluoroquinolones, aminoglycosides, and carbapenems [44]. Strains resistant to all available antimicrobial agents (pan-resistant strains) have emerged in hospitalized patients. Therefore there is an urgent need for the development of new drugs active against these pathogens [41].

P. aeruginosa and *A. baumannii* are presently among the most common MDR pathogens in hospitalized patients. Outbreaks in hospitals can be prolonged and difficult to control [6, 13, 84]. Infections caused by MDR strains are linked to higher mortality rates [6, 25]. The risk factors for MDR pathogens are previous antimicrobial therapy, hospitalization (particularly in an ICU), a high frequency of antibiotic resistance in the hospital flora, immunosuppressive therapy, and severe underlying disease [25, 48, 76]. MDR strains can spread rapidly and cause prolonged outbreaks [6, 13]. The epidemiology of these pathogens undergoes constant changes. Unexpectedly high frequencies of MDR *Acinetobacter* spp. infections (skin and soft-tissue infections, bacteremias) have been recently reported in American soldiers wounded in Iraq, even with no history of prior antibiotic therapy [10].

Emergence of MDR strains is often due to selective pressure of antimicrobial therapy. Genetic studies confirm the selection of resistant mutants and their subsequent spread [28]. Outbreaks caused by MDR *P. aeruginosa* and *Acinetobacter* spp. may follow an increased use of third-generation cephalosporins or carbapenems for therapy of infections caused by other resistant bacteria [13, 28, 48]. Until recently, imipenem was very active against clinical isolates of *P. aeruginosa* and *Acinetobacter* spp. However, imipenem resistance among these bacteria is now over 20% [38, 46]. Interestingly, it has been reported that ertapenem might have lower selective potential than other carbapenems for the emergence of carbapenem-resistant *P. aeruginosa* strains [52]. In a study by Ruiz et al. [69], resistance rates of clinical strains of *Acinetobacter* spp. against imipenem increased from 1.3% in 1991 to 80% in 1996. Many strains were also resistant to other agents, and in some serious infections colistin had to be administered as it was the only antibiotic active *in vitro*. Use of fluorochinolones also selects MDR strains of *P. aeruginosa*, including strains resistant to all antibiotics but colistin. Interestingly, levofloxacin has been linked to selective pressure, but not ciprofloxacin [66]. Recently, clinical strains resistant to all available antimicrobials have been reported [20, 39]. Several studies *in vitro* have demonstrated the synergistic effects of different antimicrobials against MDR strains of *P. aeruginosa* and *Acinetobacter* spp., but they must be evaluated *in vivo* in controlled clinical trials [20].

Due to the emergence of MDR pathogens, it is of utmost importance to develop new antimicrobial drugs. The usefulness of compounds developed earlier is also being investigated.

NEW APPROACHES TO AVAILABLE ANTIMICROBIALS

Polymyxins (polymyxin B, colistin)

Polymyxins (polymyxin B and colistin) were used in the therapy of infections in the 1970s, but due to reported toxicity and the subsequent development of less toxic drugs, their use has been discontinued. Now, with the emergence of MDR strains, their clinical use is being reconsidered [19, 41]. Several reports in the past 5 years showed that colistin toxicity is not as frequent as previously reported [20, 54]. Renal failure was rare and usually reversible, while neurotoxicity was not reported [20, 54].

Polymyxins are among the most active drugs against *Acinetobacter baumannii* and *P. aeruginosa* strains, as over 98% of isolates are susceptible to these agents [20, 34, 50, 54]. Clinical and microbiological efficacy of colistin given intravenously is 60–80% for *P. aeruginosa* and *Acinetobacter* spp. infections, except for pneumonia, where results are unsatisfactory. Aerosolized colistin has been used effectively in a few patients with nosocomial pneumonia caused by MDR *P. aeruginosa* [31]. Furthermore, colistin has been used in several cases as a salvage agent in the therapy of infections caused by strains resistant to all available antimicrobials [20, 51, 56, 61]. However, clinical strains with reduced susceptibility to polymyxin B have been reported [45].

Colistin, in combination with antibiotics from other classes, may be a useful agent for the treatment of infections caused by pandrug-resistant *P. aeruginosa* and *Acinetobacter* spp. [20]. An *in vitro* synergistic activity of colistin with imipenem and ceftazidime has been reported, but its clinical significance is unknown [29].

Aminoglycosides

Aminoglycosides are commonly used in the therapy of infections caused by Gram-negative bacilli, including *P. aeruginosa* and *Acinetobacter* spp. Results of a large ICU study comprising over 10,000 strains of *P. aeruginosa* indicated that amikacin remains the most active aminoglycoside against this pathogen [22]. Resistance to aminoglycosides among clinical isolates of *Acinetobacter* has become common [34].

Synergism between cefepim and amikacin has beeen detected against *P. aeruginosa*. In a 64-patient hospital outbreak of *P. aeruginosa* resistant to all antibiotics except colistin, colistin was used with either cefepim or amikacin, and most patients benefited [17]. A study evaluating the activity of the combination of amikacin and doxycycline in the therapy of experimental pneumonia in mice caused by *A. baumannii* showed results comparable to the efficacy of imipenem [68].

Aztreonam

Aztreonam may be used in the therapy of infections caused by *P. aeruginosa*; however, its activity against *Acinetobacter* strains is weak [5, 79]. Combination therapy of aztreonam with other antimicrobials may be effective. A two-drug (aztreonam and amikacin) and a three-drug combination (aztreonam, ceftazidime, and amikacin) were very active against MDR strains of *P. aeruginosa* in an *in vitro* study [63].

Carbapenems

Imipenem and meropenem are carbapenems commonly used in hospital practice. Many reports confirm their usefulness in the therapy of nosocomial infections caused by MDR Gram-negative bacilli. Recently published results of large studies comprising clinical strains of *Acinetobacter* spp. showed that imipenem was among the most active drugs against these bacteria [22, 34]. Interestingly, imipenem and meropenem are able to suppress *P. aeruginosa* growth even after the antibiotic level falls below the MIC (postantibiotic effect – PAE) despite the fact that β-lactams do not show PAE against other Gram-negative bacilli [14]. Apart from imipenem and meropenem, new carbapenems are being evaluated for their efficacy against MDR pathogens [23, 80].

Carbapenems may be administered as monotherapy, but with the emergence of MDR *P. aeruginosa* and *Acinetobacter* spp., combination therapies are being evaluated. *In vitro* synergism between meropenem and ciprofloxacin, as well as carbapenems and amikacin, against clinical ICU isolates of *P. aeruginosa* has been reported [18, 23]. The combinations of carbapenems and amikacin showed enhanced killing against 38–46% of tested MDR strains.

Tetracyclines

Tetracyclines (tetracycline, doxycline, minocycline) are effective *in vitro* against MDR *Acinetobacter* spp., but further research is needed to confirm their *in vivo* activity [41]. In experimental models of infection in mice and rabbits, the combination of doxycycline and rifampin showed efficacy [50]. A new glycylcyclin, tigecyclin (GAR-936), is being evaluated against clinical strains of *Acinetobacter* spp. [34, 57]. Its activity is slightly weaker than that of minocycline; however, strains resistant to minocycline are susceptible to tigecycline [34, 57]. Both these drugs are not active against *P. aeruginosa* [57].

β-Lactamase inhibitors and new cephalosporin

β-Lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) demonstrate antibacterial activity against *Acinetobacter* strains, but they do not increase the antibacterial activity of β-lactams against these pathogens. Sulbactam is superior to the other two inhibitors against clinical isolates of *Acinetobacter baumannii* and may be potentially useful in the therapy of infections caused by MDR strains [2, 3, 36, 41, 50]. Over 90% of clinical isolates of *Acinetobacter* spp. are susceptible to sulbactam [34].

A commercially available combination ampicillin/ /sulbactam can potentially be used to treat MDR *Acinetobacter* spp. infections, and its efficacy may be comparable to imipenem in the treatment of *Acinetobacter* spp. pneumonia and bacteremia [50, 60]. Co-administration of sulbactam with ampicillin or cefoperazone offers the potential of effective empirical therapy against *Acinetobacter* spp. [49]. The drug also showed synergistic activity with cefpirom [3].

Currently available β-lactamase inhibitors narrowly target only class A β-lactamases, while MDR *P. aeruginosa* strains may produce class C β-lactamases. New potent broad-spectrum β-lactamase inhibitors active against different classes of β-lactamases are currently under development [7, 42, 81]. They can possibly be combined with β-lactams. One group of novel β-lactamase inhibitors are oxapenem analogues. Their activity against MDR Gram-negative bacteria, including *P. aeruginosa*, is under evaluation [42]. A novel cephalosporin, S-3578, has been shown to be active against *P. aeruginosa* in experimental infection [78].

Rifampicin

Since clinical strains of *Acinetobacter* spp. are at present susceptible to rifampicin, addition of this agent to different therapeutic regimens is now considered in infections caused by MDR strains [34, 50]. Rifampicin and colistin showed *in vitro* synergistic effect against MDR *P. aeruginosa* [24]. In one study using a mouse model of pneumonia caused by carbapenem-resistant *A. baumannii*, rifampicin was effective and should be

considered in therapy, while colistin was not useful in these cases due to poor penetration of lung tissue [60]. Another *in vitro* study demonstrated that rifampicin combined with azithromycin was rapidly bactericidal against MDR clinical strains of *A. baumannii* [2]. Further studies are needed to evaluate the *in vivo* efficacy of rifampicin.

NOVEL APPROACHES

Macrolides

Macrolides may be useful indirectly against *P. aeruginosa* infections, even if this class of antibiotics has no significant activity against these bacteria. It has been reported that clarithromycin showed synergistic effect with tobramycin against MDR *P. aeruginosa* strains isolated from cystic fibrosis patients [70]. This combination inhibited 58% of strains. Azithromycin has an inhibitory effect on virulence factors and biofilm formation by *P. aeruginosa* [9, 27, 59, 83]. Therefore, this agent may be useful in therapy, since biofilms may contribute to antibiotic resistance, while the expression of virulence factors by this pathogen may be more important in the development of disease than replication of bacterial cells. However, further research is needed in this field.

Efflux pump inhibitors

The resistance of MDR strains may be mediated by the active export of the antibiotics out of the bacterial cell by efflux pumps. Efflux pump inhibitors are under development for use in therapy of infections with resistant strains [12].

FabI and FabK inhibitors

A major advance was recently achieved with the inhibition of enzymes mediating bacterial fatty-acid biosynthesis. Inhibitors of these enzymes represent a novel class of antibacterial agents [64]. In *P. aeruginosa* and *Acinetobacter* spp., two enzymes are involved: the enoyl-acyl carrier protein (ACP) reductase FabI and the alternative enoyl-ACP reductase FabK. Triclosan and other novel FabI- and FabK-directed inhibitors could prove to be broad-spectrum antibacterial agents, particularly for the therapy of infections caused by MDR pathogens [37, 64].

Peptide deformylase inhibitors

Another novel class of antibacterial agents, possibly lacking cross-resistance to available antibacterial drugs, are the metalloenzyme peptide deformylase inhibitors [43]. Several inhibitors have been developed to date with potent *in vitro* activity against Gram-positive and Gram-negative MDR pathogens [11].

Inhibitors of quorum sensing in P. aeruginosa biofilms

Biofilm formation is regarded as a major virulence factor of pathogenic strains of *P. aeruginosa*. Within the biofilm, highly resistant phenotypes of bacterial cells arise, so antibiotics fail to eliminate them. Furthermore, due to the heterogenicity of biofilm microenvironments, comprising variable metabolic and oxygen conditions, subpopulations of *P. aeruginosa* cells are formed [67]. It appears that anaerobic conditions within the biofilm influence the expression of exopolysaccharide production by bacterial cells. Therefore, future antimicrobial therapy may include agents combating anaerobic subpopulations of *P. aeruginosa* and quorum sensing in the biofims [32, 33, 71, 85].

BACTERIOPHAGES

Bacteriophage therapy of bacterial infections had been investigated for many years, particularly in Poland and the former Soviet Union. Their clinical use has been reviewed by Alisky et al. [1] and Sulakvelidze et al. [75]. Bacteriophages may be administered alone or in combination with antibiotics, and can be given prophylactically or as a therapy of infection. They offer many advantages, as they are very specific, replicate at the site of infection, and no serious adverse effects of their administration have been described. The selection of new phages can be accomplished within days or weeks should phage-resistant bacteria emerge. Bacteriophage therapy has now received renewed attention as a result of the emergence of MDR strains of pathogenic bacteria. Several studies have shown the efficacy of bacteriophages in the treatment of experimental infections caused by *P. aeruginosa* and *Acinetobacter* spp. in animals [72, 73]. These studies indicate that bacteriophages might also be useful in the therapy of infections caused by MDR bacterial strains in humans; however, there are no reports of recent trials emanating from this research, particularly in relation to *Acinetobacter* infections.

Therapeutic phages may cause some general symptoms due to endotoxin release from a large number of lysed bacterial cells. However, modern techniques now allow the use of non-replicating phages which kill bacteria while endotoxin release is kept to a minimum. Furthermore, genetically modified phages may be created which can deliver genes encoding proteins toxic to bacteria, and thus represent a novel approach to antimicrobial therapy [30]. Further studies are needed to assess their therapeutic use in humans.

VACCINES AND MONOCLONAL ANTIBODIES

The development of a vaccine against *P. aeruginosa* is particularly needed for cystic fibrosis patients who are frequently colonized or infected with this pathogen. Attempts at vaccines have occurred, but the search for an effective one continues, using modern techniques [8,

Table 2. Novel approaches to vaccine development against *P. aeruginosa* (based on Ramsey and Wozniak [67])

Target	Vaccine
Adherence inhibition	flagellin-based chimeric exotoxin A-pilus protein outer membrane protein (e.g. OprF) O-polysaccharide-toxin A conjugate
Type III secretion-translocation system inhibition	V antigen
Mucoid exopolysaccharide (alginate) inhibition	mucoid exopolysaccharide-alginate conjugate vaccine

53, 58, 74]. Novel approaches to vaccine development against *P. aeruginosa* have been reviewed by Ramsey and Wozniak [67] and are summarized in Table 2.

It is postulated that a vaccine should stimulate the production of antibodies that would interfere with colonization by *P. aeruginosa* and/or neutralize its virulence factors [35]. Several cell-associated and secreted antigens of this bacterium have been considered in vaccine development [35, 74]. They comprise, among others, alginate components of the extracellular slime, lipopolysaccharide (LPS), flagellar antigens, and ribosomes [74, 77]. The best studied adhesion protein which can be used in candidate vaccines is pilin [35]. Another major virulence factor of *P. aeruginosa* is exotoxin A, which is cytotoxic to mammalian cells, causing inhibition of protein synthesis and cell death. It is now possible to generate a non-toxic mutant toxin, devoid of ADP-ribosylating activity, which can be combined with other vaccine components (e.g. pilin protein, LPS) [35]. Recently, translocation proteins related to the secretive activity of *P. aeruginosa* are considered for vaccine development. Interestingly, some proteins essential for *P. aeruginosa* virulence are produced *in vivo*. Their identification by novel techniques would contribute to vaccine development [74].

Studies of several vaccines in humans have been done, including phase III clinical trials [15, 16, 47, 65, 74]. Recently the results of vaccine efficacy trails with chimeric viruses expressing *P. aeruginosa* outer membrane protein F against experimental lung infection in mice have been published [26, 82].

Administration of monoclonal antibodies against *P. aeruginosa* may be useful in the therapy of severe nosocomial infections [8]. At present they are at the preclinical phase of development. No reports on vaccine development against *Acinetobacter* spp. could be found in the literature except for a single study published over 20 years ago in Japan [62].

PREVENTIVE MEASURES

MDR strains of *P. aeruginosa* and *Acinetobacter* spp. cause outbreaks which can be prolonged and require implementation of adequate control measures [13, 21, 25, 41, 84]. Eradication of outbreaks comprises strict adherence to infection control procedures: isolation or cohorting of infected or colonized patients, hand-washing, the use of gloves and other personal protection items, proper disinfection of the hospital environment, implementation of a rational antibiotic policy, and effective antibiotic therapy of infections [13, 28, 41]. Strains of *P. aeruginosa* and *Acinetobacter* spp. readily contaminate a hospital environment and the hands of the personnel; therefore, strict hand-washing is of utmost importance [25, 28]. Special attention should be paid to the quality of the water used in the care of ICU patients [6].

CONCLUSIONS

Multidrug resistant strains of *P. aeruginosa* and *Acinetobacter* spp. have emerged in many hospitals. It is therefore crucial to implement the rational use of available antimicrobials in everyday clinical practice to prevent selective pressure and the further development of resistance in these pathogens. As MDR strains can spread within a hospital setting and cause protracted outbreaks with high mortality rates, strict infection control procedures must be observed. Novel antibiotics and novel combinations of antibiotics as well as other antimicrobial agents are under development and offer hope for effective therapy of infections caused by MDR strains of *P. aeruginosa* and *Acinetobacter* spp. The use of older agents is now also considered and investigated *in vitro*, but evaluation of their efficacy in experimental infection models and in clinical trials is urgently needed.

It is exciting to witness the fascinating development of novel approaches to the therapy of infections, particularly in the field of new classes of antimicrobials, as well as the inhibition of virulence factors of these pathogens. Investigation of the possible use of bacteriophages and vaccines in humans is in progress and in the future may supplement presently available therapeutic options.

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