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Epidemiology of infections caused by multiresistant Gram-negatives: ESBLs, MBLs, panresistant strains

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SUMMARY

Microbial drug resistance is a growing problem of global magnitude. In gram-negative pathogens, the most important resistance problems are encountered in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter*, with increasing trends observed for all major anti-gram-negative agents (β-lactams, fluoroquinolones and aminoglycosides). A matter of major concern is the emergence of new β-lactamases capable of degrading the expanded-spectrum cephalosporins and/or carbapenems, such as the extended-spectrum β-lactamases (ESBLs) and the carbapenemases. These β-lactamase genes are often associated with resistance determinants to non-β-lactam agents (e. g. aminoglycosides and fluoroquinolones), and strains producing ESBLs or carbapenemases often exhibit complex multidrug resistant phenotypes and sometimes are panresistant. The problem is worsened by the dearth of new agents active on multidrug-resistant Gram-negatives in the pipeline. The importance to develop better strategies to control resistance is underscored.

KEY WORDS: Enterobacteriaceae, *Pseudomonas aeruginosa*, *Acinetobacter*, Extended-spectrum β-lactamases, Carbapenemases, Multidrug resistance, Panresistance, Epidemiology

INTRODUCTION

Bacteria evolve so rapidly that none of the plethora of antimicrobial agents released for clinical use since the beginning of the antibiotic era have escaped from selecting resistant strains among the target pathogens. The relentless threat posed by microbial drug resistance has achieved the dimension of a global pandemic, with a relevant impact in terms of morbidity, mortality and health-care associate costs (Cosgrove, 2006).
Problems of antibiotic resistance are found in virtually any bacterial pathogen and in all epidemiological settings, although the nature and dimension of the problem can vary depending on the type of pathogen and setting. Concerning gram-negative pathogens, the major resistance challenges are encountered in *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which are among the most important causes of nosocomial infections and, for some *Enterobacteriaceae*, also an important cause of community-acquired infections. In these Gram-negatives, resistance to all active agents have been described, and clustering of multiple resistance determinants to various classes of antimicrobial agents is a common finding which results in complex multidrug resistance (MDR) phenotypes. In some cases (reported with increasing frequence) the strain becomes resistant to virtually all active agents (the so-called "panresistant" phenotype), posing a formidable challenge to antimicrobial therapy and turning back the clock to the pre-antibiotic era.

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This article reviews the most important resistance issues encountered in Gram-negative pathogens, with special emphasis on the role of the extended-spectrum β-lactamases (ESBLs) and carbapenemases as emerging resistance determinants.

EMERGING RESISTANCE ISSUES IN *ENTEROBACTERIACEAE*

The *Enterobacteriaceae* are among the most common isolates from inpatients (Styers *et al*., 2006), and *Escherichia coli* is also the most common cause of community-acquired urinary tract infections (Ronald, 2002). Recent surveillance data from intensive care units (ICUs) of hospitals in North America and in some European countries revealed that *Enterobacteriaceae* are either the most common or the second most common isolates from clinical specimens (with a prevalence varying between 29.5 and 36%) (Jones *et al.*, 2004).

The most important emerging resistance issues in *Enterobacteriaceae* are represented by resistance to fluoroquinolones and to expanded-spectrum cephalosporins, which are the workhorses for antimicrobial chemotherapy of enterobacterial infections. Although resistance to these agents can show remarkable geographic variability, resistance rates are quite high in several European countries, and increasing trends have been observed in most areas (EARSS, 2005) (Table 1).

Resistance to fluoroquinolones can be due to several mechanisms, including:

1) mutation of the topoisomerase targets causing a reduced affinity for the drugs;
2) mutational activation of efflux systems that can extrude fluoroquinolones from the cell;
3) protection of the topoisomerase target by Qnr proteins;
4) inactivation of the drug by the AAC(6')-Ib-cr variant of the common AAC(6')-Ib aminoglycoside acetyl-transferase (Hooper, 2003; Robicsek *et al*., 2006).

TABLE 1 - *Resistance rates to fluoroquinolones (FQ), 3rd generation cephalosporins (3rd GC) and carbapenems (CB) in invasive isolates of Enterobacteriaceae and P. aeruginosa from various European countries, according to the data reported by the EARSS surveillance system for year 2005 (EARSS 2005).*

	Resistance rates (%)							
Country	*E. coli*		*K. pneumoniae*			*P. aeruginosa*		
	FQ	*3rd GC*	*FQ*	*3rd GC*	*CB*	*FQ*	*CAZ*	*CB*
Bulgaria	29	28	26	50	<1	47	45	38
France	11	1	7	4	<1	27	9	14
Germany	23	2	5	6	2	22	11	24
Greece	12	7	54	61	28	39	27	39
Italy	28	8	11	20	nd	nd	nd	nd
Netherlands	10	2	6	4	<1	9	5	5
Poland	20	5	34	66	<1	31	31	27
Portugal	29	12	nd	nd	nd	nd	nd	nd
Spain	28	8	11	7	<1	14	6	17
Sweden	6	1	5	1	<1	6	5	18
UK	17	6	12	12	<1	8	3	9

The two latter mechanisms, which have been described more recently, are particularly worrisome since they are plasmid-encoded and transferable, and the corresponding resistance genes are usually carried on large plasmids bearing other clinically-relevant resistance determinants (e. g. ESBLs and AmpC-type β-lactamase genes and aminoglycoside resistance genes) (Robicsek *et al*., 2006). The epidemiological impact of these mechanisms remain to be clarified. However, their diffusion seem to be widespread (Robicsek *et al*., 2006) and recent reports from the USA revealed a remarkable prevalence of these transferable quinolone resistance genes among enterobacterial isolates showing resistance to expanded-spectrum cephalosporins and resistance or reduced susceptibility to ciprofloxacin (e. g. 20% for *qnr* genes and 16% for the *aac(6')-Ib-cr* gene in *Klebsiella pneumoniae*) (Park *et al*., 2006).

Resistance to expanded-spectrum cephalosporins is usually mediated by the production of β-lactamases. Some species, such as *Citrobacter freundii*, *Enterobacter* spp. and *Serratia marcescens*, are equipped with inducible chromosomal AmpC-like enzymes whose production can be derepressed following mutation. These so-called "derepressed mutants", known since the introduction of oxyimino-cephalosporins in clinical practice, are resistant to 3rd generation cephalosporins, cephamycins and aztreonam, but often retain susceptibility to 4th generation cephalosporins (e. g. cefepime) which are poor substrates for AmpC-like enzymes (Livermore, 1995). Since the mid 1980s, under the selective pressure generated by the use of 3rd generation cephalosporins, plasmid-mediated ESBLs capable of degrading the expanded-spectrum cephalosporins and monobactams started spreading among *Klebsiella pneumoniae*, *Escherichia coli* and other enterobacterial species. The first plasmid-mediated ESBLs were point mutant derivatives of the broad-spectrum TEM-1/2 or SHV-1 class A β-lactamases (broadly diffused among *Enterobacteriaceae* since the 1960s) that, due to mutations at some critical positions, have acquired the ability of degrading expanded-spectrum cephems and monobactams. Since the 1990s, also novel ESBLs such as the CTX-M-, PER-, GES/IBC-, and VEB-type enzymes, began to appear in clinical isolates of *Enterobacteriaceae* (Rossolini et Docquier, 2006). Of these, enzymes of the CTX-M-type have proved to be extremely successful at spreading and, in several settings, they are now the most common ESBLs in *E. coli* and *K. pneumoniae* (Livermore *et al*., 2007).

The plasmid-mediated ESBLs exhibit variable activity against 3rd and 4th generation cephalosporins, and aztreonam, while they are not active against cephamycins and carbapenems (Bradford, 2001), and production of ESBL activity results in a significant narrowing in the available therapeutic options. There is broad evidence that the use of expanded-spectrum cephalosporins for treatment of ESBL producers is associated to high failure rates even if MICs of these compounds remain relatively low-level, while ESBL producers often exhibit MDR phenotypes that include fluoroquinolones and aminoglycosides (Paterson and Bonomo, 2005). In fact, carbapenems are considered the most reliable agents for treatment of serious infections caused by ESBL producers (Paterson et Bonomo, 2005), while a poorer outcome has been reported with fluoroquinolones even if the ESBL-producing strains retained in vitro susceptibility to these drugs (Endimiani *et al.*, 2004). Infections caused by ESBL producers are associated with higher morbidity and mortality, a higher risk for inadequate treatment, and increased health-care associated costs (Schwaber *et al*., 2006).

Although the ESBLs are currently the most widespread and prevalent mechanism of acquired resistance to expanded-spectrum β-lactams in *Enterobacteriaceae*, additional β-lactamases, namely the plasmid-mediated AmpC-type β-lactamases and the carbapenemases, are also are also emerging in some settings. Production of the former enzymes can be suspected in isolates showing an MDR phenotype including 3rd generation cephalosporins and cephamycins but not cefepime (Livermore, 1995). Their impact remains overall lower than that of ESBLs, but moderate to high rates of AmpC-type β-lactamase production have been recently reported among ceftazidime-resistant isolates of *E. coli* and *K. pneumoniae* from the USA and Taiwan, respectively (Alvarez *et al*., 2004; Yan *et al*., 2006). Acquired carbapenemases emerging in *Enterobacteriaceae* include the KPC-, IMI- and SME-type serine-β-lactamases and the IMP- and VIM-type metallo-β-lactamases. Strains pro-

ducing serine carbapenemases are spreading mostly in the East coast of the USA (Desphande *et al.*, 2006), while those producing metalloenzymes are still quite uncommon except in Greece, where a consistent dissemination of strains producing VIM-type enzymes has been reported (Giakkoupi *et al.*, 2003; Ikonomidis *et al.*, 2005; Galani *et al.*, 2007), which is apparently the major cause of the very high resistance rates (27.9% in 2006) to carbapenems observed in *K. pneumoniae* from that country (http://www.earss.rivm.nl). Clustering of ESBL and MBL determinants has also been reported in strains of *K. pneumoniae* showing a panresistant phenotype (Miriagou *et al.*, 2005).

EMERGING RESISTANCE ISSUES IN GRAM-NEGATIVE NONFERMENTERS

P. aeruginosa is one of the most important nosocomial pathogens, being a major cause of pneumonia, bacteremia, and urinary tract infections (Pier et Ramphal, 2005).

The latest global surveillance data from the SENTRY surveillance system, referred to more than 8000 clinical isolates collected during the period 2001-2004 from the Americas, Europe and the Asia-Pacific region showed that only polymyxin B remains active against the vast majority of isolates, while for other anti-pseudomonas susceptibility rates ranging from 70% (ciprofloxacin) to 88% (amikacin) were observed (Figure 1). EARSS data, which since 2005 also cover surveillance of invasive isolates of *P. aeruginosa*, revealed even higher resistance rates in some countries, especially in the Mediterranean area and Eastern Europe (Table 1).

In *P. aeruginosa*, emerging resistance issues affect all the major anti-pseudomonal agents including β-lactams, fluoroquinolones and aminoglycosides. Concerning fluoroquinolones, *P. aeruginosa* can become resistant to these agents by two major mutational mechanisms: topoisomerase target modifications, and overexpression of a number of multidrug efflux systems of the RND family such as MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM (Hooper, 2003). The efflux systems can also extrude β-lactams (MexAB-OprM, MexCD-OprJ, and MexXY-OprM) and aminoglycosides (MexXY-OprM), so that an MDR phenotype can be selected for in a single mutational step (Rossolini et Mantengoli, 2005). Resistance to anti-pseudomonal β-lactams can also arise by additional mechanisms. Decreased outer membrane permeability due to mutations reducing the amount of the OprD porin, which is the preferential entry channel for carbapenems, is an important cause of decreased susceptibility to those compounds (Livermore, 2001). Mutants showing reduced production of OprD are easily selected in the presence of carbapenems, and this represents the major mechanism accounting for the tight relationship existing between carbapenem consumption and emergence carbapenem resistance in *P. aeruginosa* (Lepper *et al.*, 2002). β-Lactamase production is another important mechanism of acquired β-lac-

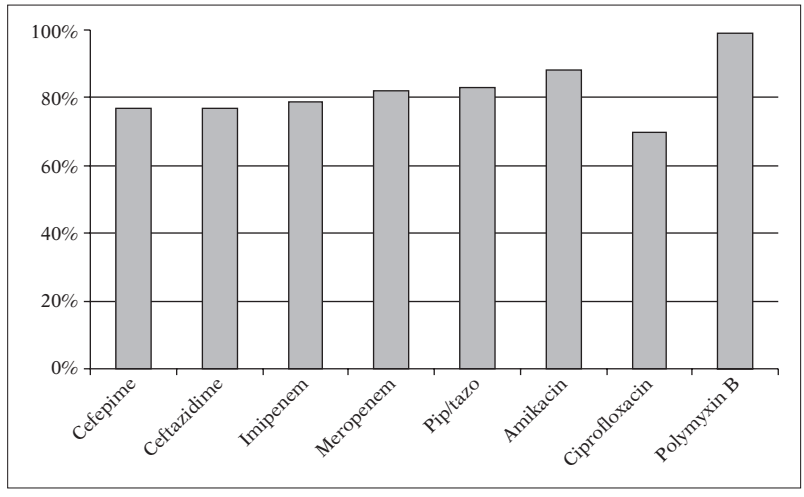

FIGURE 1 - *Global surveillance data on susceptibility rates to various agents of* P. aeruginosa *isolates, reported by the SENTRY surveillance system (Gales et al., 2006).*

tam resistance in *P. aeruginosa*. The species is equipped with a chromosomally-encoded AmpC-type β-lactamase whose derepressed hyperproduction can be responsible for resistance to ceftazidime and anti-pseudomonas penicillins and, depending on the amount of enzyme and/or the simultaneous presence of a permeability defect, it can also contribute to decreased susceptibility to cefepime and imipenem (Livermore, 1995). In addition, *P. aeruginosa* can acquire, by horizontal gene transfer, a broad repertoire of secondary β-lactamases, encoded by mobile genetic elements, which can variably contribute to the β-lactam resistance phenotype depending on their substrate specificity. Acquired β-lactamases that can be encountered in *P. aeruginosa* include:

1) narrow-spectrum penicillinases (e. g. PSE-1, PSE-4 and some OXA-type enzymes), which confer resistance to penicillins and cefoperazone;
2) ESBLs (e. g. PER-, VEB-, GES- and some OXA-type), that can confer resistance to penicillins, cephalosporins and aztreonam;
3) MBLs (of the IMP-, VIM-, SPM-, and GIM-type), which have an exceedingly broad substrate specificity, including carbapenems, and are not susceptible to any of the currently available β-lactamase inhibitors, and as such are the most challenging (Rossolini and Mantengoli, 2005; Rossolini, 2005).

Most genes encoding MBLs are carried on mobile gene cassettes inserted into integrons, where they are usually associated to other types of resistance genes: this explains why MBL production is usually associated with complex MDR phenotypes (Walsh *et al.*, 2005). Although the overall prevalence of *P. aeruginosa* producing ESBLs or MBLs remains relatively low, several outbreaks caused by MDR *P. aeruginosa* producing acquired ESBLs or MBLs have recently been reported and are a matter of increasing clinical concern (Rossolini, 2005).

In fact, in addition to developing resistance to single agents, *P. aeruginosa* exhibits a remarkable attitude to evolve MDR phenotypes, and an increasing trend has been reported for multidrug resistance rates in recent years (Obritsch *et al.*, 2004). In some cases, resistance can affect virtually all anti-pseudomonal agents resulting in the so-called "panresistant" phenotypes (Livermore, 2002; Bonomo and Szabo, 2006) that are exceedingly difficult to treat and lead us back to conditions reminiscent of the pre-antibiotic era.

Acinetobacter baumannii is an important opportunistic pathogen that can survive for long periods in the hospital environment and cause nosocomial infections in severely debilitated patients, especially in the ICU setting, where acinetobacters can be an important causes of hospital-acquired pneumonia and bacteremia associated to a poor prognosis (Bergogne-Berezin and Towner, 1996). *Acinetobacter* outbreaks have become a problem in several European hospitals, and the impact of these outbreaks can be magnified by the remarkable trend of this pathogen to acquire resistance to multiple antimicrobial agents (Van Looveren and Goossens, 2004).

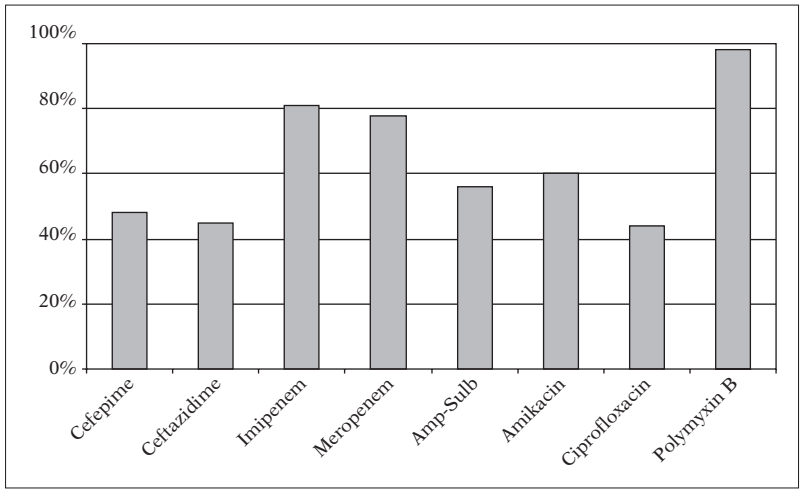

FIGURE 2 - *Global surveillance data on susceptibility rates to various agents of* Acinetobacter *spp. isolates, reported by the SENTRY surveillance system (Gales et al., 2006).*

TABLE 2 - *Susceptibility rates to several anti-acinetobacter agents reported for isolates from ICUs of hospitals in North America and some European countries reported by the TSN database for years 2000-2002 (Jones et al., 2004).*

Antimicrobial agent	*Susceptibility rates (%) in*				
	USA	*Canada*	*France*	*Germany*	*Italy*
Cefepime	44	67	28	74	18
Cefotaxime	23	37	15	35	11
Ceftazidime	42	71	35	67	26
Ciprofloxacin	40	72	38	75	21
Gentamicin	47	73	49	82	23
Imipenem	87	96	94	96	78
Meropenem	66	94	68	96	75

The latest global surveillance data from the SENTRY surveillance system, referred to more than 2500 clinical isolates collected during the period 2001-2004 from the Americas, Europe and the Asia-Pacific region showed that, similar to what observed for *P. aeruginosa*, only polymyxin B remains active against the vast majority of isolates, while for other anti-acinetobacter agents susceptibility rates ranging from 44% (ciprofloxacin) to 81% (imipenem) were observed, with only carbapenems retaining substantial activity (Figure 2).

Recent surveillance data for *Acinetobacter* isolates from ICUs of hospitals in North America and in some European countries revealed susceptibility rates that, for most agents, were even lower, with a remarkable geographic variability (Table 2). This variability likely reflects the fact that *Acinetobacter* typically disseminate by clonal spread, with clones that display different resistance mechanisms spreading in different settings. In fact, the introduction of drug-resistant clones in the hospital setting can rapidly modify the local hospital epidemiology (Corbella *et al.*, 2000; Poirel *et al.*, 2003).

Since carbapenems are among the few anti-acinetobacter agents which retain consistent activity, the emergence of carbapenem-resistant *Acinetobacter* strains is a matter of great concern. Several mechanisms can be exploited by *Acinetobacter* to develop carbapenem resistance. The best known are represented by the production of acquired carbapenemases (either metallo-enzymes of the IMP and VIM type, or enzymes of the OXA-23, OXA-24 and OXA-58 type), or the overexpression of the resident OXA-51-like β-lactamase, although altered PBP targets, and reduced permeability or efflux systems could also play a role (Poirel and Nordmann, 2006). As with *P. aeruginosa*, carbapenem use is a powerful selector for carbapenem-resistant *Acinetobacter* clones which, once established in the hospital setting, can prove very difficult to eliminate (Corbella *et al.*, 2000; Poirel *et al.*, 2003; Urban *et al.*, 2003). Similar to *P. aeruginosa* multidrug resistance is a common feature among *Acinetobacter* clones and panresistance sometimes occur (Bonomo and Szabo, 2006). It is now clear that with these two gram-negative pathogens we are closer to the "end of antibiotics" than with any other gram-negative or gram-positive bacterial pathogen.

CONCLUDING REMARKS

The global diffusion of antibiotic resistance, with the emergence of complex MDR and panresistant phenotypes, is now a matter of major concern with gram-negative pathogens.

Systematic and capillary surveillance is very important to monitor the phenomenon, which can rapidly evolve due to the dissemination of MDR clones and/or plasmids carrying multiple

resistance genes in the clinical setting. Surveillance data are essential for adequate selection of empiric therapy for serious infections, and to monitor the outcome of interventions aimed at infection and resistance control.

The dearth of new drugs for MDR Gram-negatives in the pipeline underscore the need to reinforce research in this area, and to develop better strategies to control the dissemination of resistance in the clinical setting.

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