Epidemiology of multi-resistance Gram negative pathogen circulating in Liguria and molecular characterization of different carbapenemases

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SUMMARY

This study was conducted during January-April 2010 with the collaboration of 7 clinical microbiology laboratories evenly distributed across the Ligurian area to identify the most frequent Gram negative species and to evaluate their antibiotic susceptibility patterns

Overall, 110 consecutive multi-resistant non duplicate Gram negative isolates, were collected and sent to the co-ordinating laboratory (Sezione di Microbiologia del DISC, University of Genoa, Italy) together with susceptibility data obtained by routine methods. In addition, strains resistant to carbapenems were characterized by PCR.

A total of 110 Gram negative multi-resistance strains were found, including 74 and 36 isolated from healthcare or nosocomial settings and community acquired infections, respectively.

The most represented pathogens were: *A. baumannii* (38, 34.5%), *E. coli* (30, 27.2%), *P. aeruginosa* (29, 26.3%), *K. pneumoniae* (9, 8.2%) and *P. mirabilis* (4, 3.6%). *A. baumannii* were more frequently collected from healthcare settings or nosocomial samples, while the other strains were generally equally isolated from in- and out-patients.

Amikacin was the most active molecule against *E. coli* and *P. mirabilis* (96,7% and 100% of susceptible stains respectively). Colistin was the only active molecule agains *A. baumanii* and *P. aeruginosa* (100% of susceptible strains). Against *K. pneumoniae* tigecycline and colistin were the most active molecules (100% of susceptible strains).

Imipenem was the most active compound against *E. coli* and *P. mirabilis* (100% of susceptible strains). A large number (97.4%) of *A. baumannii* was resistant to imipenem. *K. pneumoniae* and *P. aeruginosa* showed rates of resistance of 88% and 34.4% respectively.

A. baumannii, K. pneumoniae and P. aeruginosa isolates resistant to Imipenem, carried OXA-23, KPC and VIM carbapenemases. These data shown a significant spread of multidrug-resistant Gram negative bacteria in hospitals and in communities. The production of carbapenemase in *A. baumannii, K. pneumoniae* and *P. aeruginosa* is now an important phenomenon in our region.

INTRODUCTION

Multidrug-resistant Gram negative bacilli are serious problem in healthcare facilities. These organisms rapresent difficult treatment challenges, and limiting their dissemination is an important public health objective. In 2010, infections caused by multidrug-resistant bacteria simultaneously resistant to at least tree different classes of antimicrobial agents, MDR continue to challenge physicians and endanger their patients' lives (7).

There are a number of different methods in which Gram-negative organisms develop resistance to antibiotics: β-lactamase production is the most important.

Extended spectrum β-lactamase (ESBLs) are most often found in *Klebsiella pneumoniae* and *Escherichia coli*; however, other Gram negative bacilli including *Enterobacter* spp., *Pseudomonas aeruginosa*, *Citrobacter freundii, Morganella morganii* and *Serratia marcescens* have also been found to produce these enzymes as well as AmpC cephalosporines (4).

Carbapenems are the preferred antimicrobial

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agents for ESBL and AmpC-producing organisms; however, their widespread use in outbreaks and endemic regions of these organisms has led to increased rates of carbapenem-resistant *P. aeruginosa* and *Acinetobacter* spp. (8).

A mechanism of carbapenem resistance in *Enterobacteriacee* occurs by the production of â-lactamases that hydrolys carbapenem compounds. These include the serine β-carbapenemase (KPC, Ambler class A enzymes), metallo β-lactamases (MBLs, Ambler class B) and extended spectrum oxacillinases (OXA, Ambler class D).

This survey was planned to identify the most frequent MDR species and to evaluate their antibiotic susceptibility patterns among Gram negative bacteria collected from clinical samples in Liguria. In addition, strains resistant to carbapenems (drugs of last resort against Gram negative) were characterized by PCR.

MATERIAL AND METHODS

Bacterial isolates

This study was conducted during January 2010-April 2010 with the collaboration of 7 clinical microbiology laboratories evenly distributed across the Ligurian area. The enrolled Laboratories were: ASL 1 Imperiese, Imperia; ASL 3 San Carlo Hospital, Genoa-Voltri; Ente Ospedaliero Galliera Hospital, Genoa; International Evangelical Hospital, Genoa; Istituto Giannina Gaslini, Genoa; ASL 4 Chiavarese, Genoa; ASL22, Santa Corona Hospital, Pietra Ligure (Savona).

Overall, 110 consecutive multi-resistance Gram negative isolates, belonging to the *Enterobacteriaceae* family and other non-fermenting bacteria, were collected and sent to the co-ordinating laboratory (Sezione di Microbiologia del DISC, University of Genoa, Italy). Strains isolated from any type of specimen, from in- and out-patients were studied, with the exception of duplicate strains from the same patient. Participating laboratories also provided susceptibility data obtained by their routine method.

Antimicrobial susceptibility testing

Minimum inhibitory concentrations of imipenem were determined by the broth microdilution method suggested by Clinical Laboratory Standard Institute (CLSI) (12).

Escherichia coli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls strains.

PCR amplification

For strains with reduced susceptibility to imipenem (MIC> 0.5 mg\L) molecular analysis were performed to detect genes responsible for resistance. DNA was extracted from isolates by boiling one colony in 250 ìL of sterile ultrapure water for 10 minutes, followed by cooling in ice for 10 minutes and centrifugation for 1 min at 14,000 rpm. Supranatant were conserved at -20°C until amplification.

VIM, OXA-23 and KPC carbapenemases was identified by PCR as previously described (1, 3, 16).

RESULTS

Table 1 summarises the complete list and the distribution of the pathogens collected in this study. A total of 110 Gram negative multi-resistance strains were found, including 74 and 36 isolated from healthcare settings or nosocomial-and community acquired infections, respectively.

Most represented pathogens were: A. *baumannii* (38, 34.5%), *E. coli* (30, 27.2%), *P. aeruginosa* (29, 26.3%), *K. pneumoniae* (9, 8.1%) and *P. mirabilis* (4, 3.6%). *A. baumannii* strains were more frequently isolated from healthcare settings or nosocomial patients.

Nosocomial samples were collected mainly from patients hospitalised in Intensive Care Unit (27%) (Table 2) and general medicine wards (21.6%), in 7 cases the samples were from patients living in healthcare settings (9.4%).

Bacterial isolates were obtained from urine (48, 43.6%), broncho-aspirate (27, 24.5%), *sputum* (11, 10%), skin wound (9, 8.2%), catheter urine (4, 3.6%), pharyngeal swab (3, 2.7%) and other sites (8, 7.3%).

The antibiotics susceptibility patterns of the strains collected from the various centres is displayed in Table 3.

Amikacin was the most active molecule against *E. coli* (96.7% of susceptible strains) followed by gentamicin (60% of susceptible strains), trimethoprim-sulfamethxazole (43,3% of susceptible strains) e amoxicillin-clavulanic acid (43.3%). The other antibiotics (piperacillin-tazobactam, ceftazidime, third generation cephalosporins and ciprofloxacin) showed rates of resistance higher than 80%.

Colistin was the most active molecule against *A. baumannii* (100% of susceptible strains), followed by amikacin (63.1% of susceptible strains). The other molecules (gentamicin, piperacillin-tazobactam, third generation cephalosporins, ciprofloxacin, amoxicillin-clavulanic acid and trimethoprim-sulfamethxazole) were not active (100% of resistant strains).

Tigeciline and colistine were the most active molecules against *K. pneumoniae* (100% susceptibility), followed by gentamicin (88.8% of susceptible strains). Amikacin, piperacillin-tazobactam, third generation cephalosporins, ciprofloxacin, ceftazidime and trimethoprim-sulfamethxazole were not active against *K. pneumoniae*.

All *P. mirabilis* strains were suscetible to amikacin, the other molecules (gentamicin, piperacillin-tazobactam, third generation cephalosporins, ciprofloxacin, amoxicillin-clavulanic acid, ceftazidime and trimethoprim-sulfamethxazole showed rate of resistence higher than 75%.

Colistin was the most effective molecule against *P. aeruginosa* (100% of susceptible strains), followed by amikacin (58.6% of susceptible strains). Gentamicin, piperacillin-tazobactam, third generation cephalosporins, ciprofloxacin, amoxicillin-clavulanic acid and trimethoprim-sulfamethxazole showed rate of resistence higher than 45%.

Imipenem was the most active compound against *E. coli* and *P. mirabilis* (100% of susceptible strains) 97.14% of *A. baumannii* was resistant to imipenem *K. pneumoniae* and *P. aeruginosa* showed rate of resistance respectively 88% and 34.4%.

Imipenem resistant *A. baumannii, K. pneumoniae* and *P. aeruginosa* isolates carried OXA-23, KPC and VIM carbapenemases, respectively.

Table 1. *Distribution of the strains collected in this survey according to their origin*

	Number and origin			
Strains	Nos-HC	Com	Tot	%
A. baumannii	36	2	38	34.5
E. coli	14	16	30	27.2
P. aeruginosa	16	13	29	26.3
K. pneumoniae	8	1	9	8.1
P. mirabilis	1	3	4	3.6
Total	75	35	110	
%	68.2	31.8		

Nos-HC, nosocomial- healthcare- acquired; Com, community-acquired, Tot, total

DISCUSSION

This report described the epidemiology of different MDR bacteria carring infections in Liguria and their antibiotic susceptibility patterns, evaluating samples from over 110 patients recruited by 7 hospitals.

The need for local, national or international surveillance to evaluate the rate of bacterial resistance to antibiotics, is generally suggested in order to chose the best drug in empiric therapy, and to gain information about the emerging pathogens and their identification, as well as evolution toward resistance to the more frequently used antimicrobials (5, 6, 9, 10, 11, 15).

Although often thought of as a problem for critically ill patients in acute care hospitals, MDR Gram negative bacilli are increasing being found outside of these circumstances. Patients in long term care facilities appear to be at risk for infection or colonization with multi-resistant Gram negative bacilli (13, 14).

A. baumannii was the most more frequent

Table 2. *Distribution of nosocomial strains according to the different clinical settings*

	Number of strains collected							
Strains	MED	ICU	ID	NEU	PNE	HCS	OTHER	UNK
A. baumannii (36\74)	5	10	2	1		2	9	7
E. coli (14\74)	6	1	1		2	3		1
P. aeruginosa (16/74)	5	5	1				2	3
K. pneumoniae (8/74)		5		1		1		1
P. mirabilis (1/74)						1		
Total	16	21	4	2	2	7	11	12

Med, Medicine; ICU, Intensive Care Unit; ID,Infectious Diseases; Neu, Neurology; Pne, Pneumology; HCS, Healthcare setting; Unk, unknown.

Table 3. *Percentages of susceptibility to major classes of antibiotics*

	AMK	**GM**	**AMC**	**PIP\TAZ**	**CAZ**	**III generation cephalosporins**	**CIP**	**SXT**	**COL**	**TIGE**	**IMI**
A. baumannii (38/110)	63.1% (24/38)	0% (0/38)	0% (0/38)	0% (0/38)	nt	0% (0/38)	0% (0/38)	0% (0/38)	100% (38/38)	nt	2.6% (1/38)
E. coli (30/110)	96.7% (29/30)	60.00% (18/30)	43.3% (13/30)	20% (6\30)	13.30% (4/30)	13.30% (4/30)	0.00% (0/30)	43.3% (13/30)	nt	nt	96.7% (29\30)
P. aeruginosa (29/110)	58.6% (17/29)	6.8% (2/29)	48.3% (14/29)	41.4% (12/29)	nt	34.5% (10/29)	3.40% (1/29)	17.2% (5/29)	100% (29/29)	nt	65,5% (19/29)
K. pneumoniae (9/110)	0% (0/9)	88.8% (8/9)	nt	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	0% (0/9)	100% (9/9)	100% (9/9)	22% (2/9)
P. mirabilis (4/110)	100% (4/4)	25% (1/4)	25% (1/4)	50% (2/4)	25% (1/4)	25% (1/4)	25% (1/4)	25% (1/4)	100% (4/4)	nt	nt

AMK: amikacin, GM: gentamicin, AMC: amoxicillin-clavulanic acid, PIP\TAZ: piperacillin\tazobactam, CAZ: ceftazidime, CIP: Ciprofloxacin, SXT: trimethoprim-sulfamethxazole, COL: colistine, TIG: Tigeciclina, IMI: Imipenem.

pathogen isolated from ICU patients which accounted for 47.61% of all isolates collected in these wards, indicating the increasing importance of this species among infections diagnosed in this settings (2, 17). The 36 *A. baumannii* nosocomial isolates in this study showed high level of resistance to the great majority of the antibiotics, only 1 strain was found susceptible to imipenem and 24 strains were susceptible to amikacin. Only colistin was active against *A. baumannii* (100% of susceptible strains).

Finally, carbapenemase production in *A. baumannii, K. pneumoniae* and *P. aeruginosa* rappresent an important resistance threat in our region.

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