Prevalence of 16S rRNA methylase genes in *Acinetobacter baumannii* isolates in Greece in a three year period

Galani I, Chatzikonstantinou M, Katsala D, Petrikkos G, Souli M.

4th Dept of Internal Medicine, Athens University School of Medicine, Athens, Greece

National and Kapodistrian University of Athens

ABSTRACT

Objectives: Since 2011, a continuous increase in resistance rates of aminoglycosides, mostly of tobramycin, in ICU and medical wards, among *Acinetobacter baumannii* isolates was observed in data presented by Whonet Greece. The aim of this study was to investigate the prevalence of 16S rRNA methylase genes in consecutively collected *A. baumannii* isolates in Athens, Greece.

Methods: *A. baumannii* isolates resistant to both amikacin and gentamicin (n=243), consecutively collected during a three year period (2010 - 2012) in our Infectious Diseases Laboratory, were tested further for MIC determination to amikacin, gentamicin, tobramycin, netilmicin, apramycin and neomycin with the broth dilution technique. Isolates with MICs >=512 mg/L to at least the first four aminoglycosides were examined for the presence of 16S rRNA methylase genes (*armA, rmtB, rmtC, rmtA, rmtD* and *npmA*) by PCR. Molecular typing was performed by REP-PCR. Carbapenemase production was confirmed by PCR.

Results: Fifty five (22.6%) *A. baumannii* isolates were positive for *armA* and highly resistant to all clinically used aminoglycosides tested (MICs >=512 mg/L). Apramycin MICs ranged from 32 to 64mg/L, with an MIC50 of 32 and an MIC90of 64mg/L. Neomycin MICs ranged from 4->512, with both MIC50 and MIC90 >512mg/L. All *armA*-bearing *A. baumannii* strains were OXA-23 producers with 94.6% of them being only susceptible to colistin. All *armA - A. baumannii* isolates belonged to the same clone. The prevalence of *armA*-positive *A. baumannii* isolates were 0%, 19.7% and 86.2% in the years 2010 – 2011 – 2012 respectively.

Conclusions: Our data demonstrate an increasing prevalence of *armA*-positive *A. baumannii* isolates since 2010 and a strong connection to OXA-23 carbapenemase. OXA-23-producing *A. baumannii* was first reported in 2012 from clinical specimens taken in 2010 and 2011 at the University Hospital of Larissa in central Greece, while since 2009 the predominant oxacillinase among carbapenem-resistant *A. baumannii* in Greek hospitals was the OXA-58. The spread of one clone of XDR isolate producing both carbapenemase OXA-23 and 16S rRNA methylases raises clinical concern and may become a major therapeutic threat in the future.

CONTACT

Irene Galani
National and Kapodistrian University of Athens,
Faculty of Medicine
4th Department of Internal Medicine,
Infectious Diseases Laboratory
Email: egalani@med.uoa.gr

INTRODUCTION

- Since 2011, a continuous increase in resistance rates of aminoglycosides, mostly of tobramycin, in ICU and medical wards, among *Acinetobacter baumannii* isolates was observed in data presented by Whonet Greece.
- *A. baumannii* has a considerable capacity to acquire mechanisms conferring resistance to broad-spectrum *β*-lactams, carbapenems, aminoglycosides and fluoroquinolones.
- Resistance to carbapenems in *A. baumannii* is mainly mediated by the acquisition of class D carbapenemase-encoding genes, with $bla_{\text{OXA-23-like}}$ being the most frequently identified carbapenemase-encoding gene.
- In Greece the predominance of OXA-58-producers among carbapenem-resistant *Acinetobacter baumannii* has been documented in Greek hospitals till 2011. The isolation of OXA-23 - producing *A. baumannii* from clinical specimens taken in 2010 and 2011 was first reported at a University Hospital of central Greece.
- The occurrence of genes encoding aminoglycoside-modifying enzymes, possibly as gene cassettes in class 1 integrons, is the main mechanism of resistance to aminoglycosides. In addition, 16S rRNA methylases conferring high-level pan-aminoglycoside resistance have recently been identified in *A. baumannii*, including OXA-23-producing isolates.
- The aim of this study was to investigate the prevalence of 16S rRNA methylase genes in consecutively collected *A. baumannii* isolates in Athens, Greece.

MATERIAL AND METHODS

- Two hundred and forty-three (n=243) *A. baumannii* isolates resistant to both amikacin and gentamicin consecutively collected during a three year period (2010-2012) in our Infectious Diseases Laboratory, were tested further.
- Only one isolate per patient was included in the study.
- Species identification of isolated bacteria and primary MIC determinations were performed using an automated system (BD Phoenix automated microbiology system; BD Diagnostic Systems).
- MIC determinations to amikacin, gentamicin, tobramycin, netilmicin, apramycin and neomycin were performed with the broth microdilution technique.
- Isolates with MICs ≥512 mg/L to the first four of the above aminoglycosides were examined for the presence of 16S rRNA methylase genes (*armA, rmtA, rmtB, rmtC, rmtD* and *npmA*) by multiplex PCR (Doi & Arakawa 2007; Berçot et al 2011).
- Genomic fingerprinting was carried out by repetitive-element PCR (rep-PCR) analysis (Versalovic et al 1991).
- Metallo-β-lactamase activity was detected phenotypically using the imipenem-EDTA combined disc test. OXA carbapenemase production was confirmed by multiplex PCR (Woodford et al 2006).
- Sequencing of the PCR products were performed by MWG-THE Genomic Company.

RESULTS

- Fifty five (22.6%) *A. baumannii* isolates were positive for *armA* and highly resistant to all clinically used aminoglycosides tested (MICs >=512 mg/L) (Chart 1, Figure 3).
- Apramycin MICs ranged from 32 to 64mg/L, with an MIC_{50} of 32 and an MIC_{90} of 64mg/L (Table 1).
- Neomycin MICs ranged from 4->512, with both MIC_{50} and MIC_{90} >512mg/L (Table 1).
- All *armA*- bearing *A. baumannii* strains were OXA-23 producers with 94.6% of them susceptible only to colistin (Figure 2).
- All *armA* - *A. baumannii* isolates belonged to the same clone (Figure 1).
- The prevalence of *armA*-positive *A. baumannii* isolates were 0%, 19.7% and 86.2% in the years 2010 – 2011 – 2012 respectively (Figure 1).

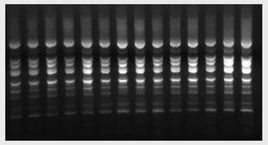

Figure 1. Clonality of *armA* – bearing *A. baumannii* isolates.

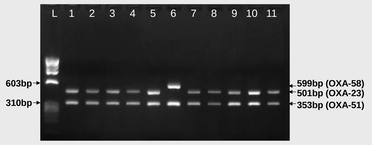

Figure 2. OXA carbapenemase detection by multiplex PCR.

Figure 3. 16S rRNA methylase detection by multiplex PCR.
Lanes 1-5 & 8-11: armA positive isolates
Lanes 6 & 7: 16S RMT negative isolates

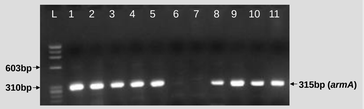

Table 1. Aminoglycoside susceptibilities evaluated by broth microdilution method. Isolates included in the study were primarily resistant to amikacin (>32) and gentamicin (>8) to Phoenix G Neg Panel NMIC/ID-94

Aminoglycoside	Isolates	MIC Range	MIC_{50}	MIC_{90}	%S
Amikacin	All	8-1024	1024	1024	1.10%
Gentamicin	All	4-1024	256	1024	0.74%
Tobramycin	All	0.5-1024	64	1024	26.94%
Netilmicin	All	4-1024	1024	1024	19.93%
Apramycin	All	2-1024	16	64	ND
	armA (+)	32 - 64	32	64	ND
Neomycin	All	1-1024	64	1024	ND
	armA (+)	4 - >512	>512	>512	ND

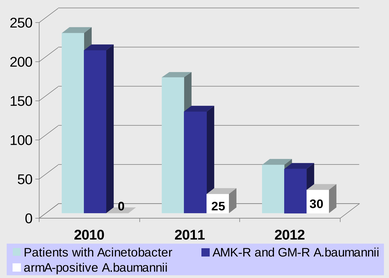

Chart 1. *Acinetobacter baumannii* isolates tested

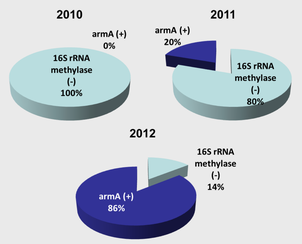

Chart 2. Prevalence of *armA*-bearing *A.baumannii* isolates

DISCUSSION

During 1999 to 2009, *A. baumannii* strains carrying the $bla_{\text{OXA-58}}$ carbapenemase gene predominated among carbapenem-resistant isolates of this species in the hospital flora in Greece as well as in other Mediterranean countries.

Since 2009, isolation of *A. baumannii* producing the OXA-23 carbapenemase has been increasingly reported in European countries and a massive 'replacement' of OXA-58 *A. baumannii* by OXA-23 producers has been described in Italian hospitals [D'Arezzo et al 2011].

In Greece, the first description of *A. baumannii* strains producing OXA-23 was reported in a tertiary care hospital in central Greece where during 2011, they rapidly 'replaced' the previously predominant OXA-58 - producing *A. baumannii* isolates [Liakopoulos et al 2012]. The isolates were also resistant to aminoglycosides and fluoroquinolones and susceptible only to colistin.

The hypothesis that the higher carbapenem and aminoglycoside MICs of the $bla_{\text{OXA-23}}$ - *armA* positive isolates may provide, a selective advantage in the hospital setting has to be investigated.

A study involving a number of hospitals in different parts of Greece would provide a comprehensive picture regarding the *A. baumannii* strains circulating in our country.

CONCLUSIONS

- Our data demonstrate an increasing prevalence of *armA*-positive *A. baumannii* isolates since 2010 and a strong connection to OXA-23 carbapenemase.
- OXA-23 - producing *A. baumannii* was first reported in 2012 from clinical specimens taken in 2010 and 2011 at the University Hospital of Larissa in central Greece, while before that the predominant oxacillinase among carbapenem-resistant *A. baumannii* in Greek hospitals was the OXA-58.
- The spread of one clone of XDR isolate producing both carbapenemase OXA-23 and 16S rRNA methylases raises clinical concern and may become a major therapeutic threat in the future.

REFERENCES

1. Wachino J, Arakawa Y. Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: an update. Drug Resist Updat. 2012;15:133-48.
2. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res. 1991; 19: 6823-31.
3. Woodford N, Ellington MJ, Coelho JM et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. International Journal of Antimicrobial Agents 27 () 351–353
4. Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis 2007;45:88-94
5. Berçot B, Poirel L, Nordmann P. Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM-1 producers. Diagn Microbiol Infect Dis. 2011; 71:442-5.
6. D'Arezzo S, Principe L, Capone A, Petrosillo N, Petrucca A, Visca P. Changing carbapenemase gene pattern in an epidemic multidrug-resistant Acinetobacter baumannii lineage causing multiple outbreaks in central Italy. J Antimicrob Chemother 2011;66:54-61
7. Liakopoulos A, Miriagou V, Katsifas EA, Karagouni AD, Daikos GL, Tzouvelekis LS, Petinaki E. Identification of OXA-23-producing Acinetobacter baumannii in Greece, 2010 to 2011. Euro Surveill. 2012 Mar 15;17(11).