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Short Communication

Emergence of carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals

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SUMMARY

Background: Healthcare-associated infections are a worldwide threat to hospitalized patients, especially those in intensive care units. The prevalence of these infections in Egypt, and their antimicrobial resistance patterns and mechanisms, were investigated in this study.

Methods: A total of 547 cases of healthcare-associated infections were investigated. Causative agents were identified and antimicrobial susceptibility determined. Carbapenem-resistant *Acinetobacter baumannii* isolates were further investigated for their resistance mechanism via the modified Hodge test, inhibitor-potentiated disk diffusion test, synergy with carbonyl cyanide chlorophenylhydrazone, and PCR. Moreover, clonal linkage was examined via enterobacterial repetitive intergenic consensus (ERIC)-PCR.

Results: *Klebsiella spp* was the most prevalent species in the isolates examined (217; 40%). Although *A. baumannii* represented only 10% of the total isolates, it showed the highest percentage of carbapenem resistance (74%). PCR showed that 100% of the resistant isolates carried both *bla*_{OXA-51} and *bla*_{OXA-23} genes, 85% carried the class 1 integrase genes, and only 2.5% carried metallo-beta-lactamase (*bla*_{VIM}). ERIC-PCR indicated that isolates from different hospitals were genetically linked.

Conclusions: These findings represent the first report of the alarming spread of OXA-23 carbapenemase in *A. baumannii* in Egyptian intensive care units. The spread of such strains has serious health consequences and requires the application of strict infection control measures.

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1. Introduction

Healthcare-associated infections (HAIs) occur worldwide, with significant economic costs and mortality. For decades, Gram-negative bacilli (GNB) have maintained their share of HAIs; however *Acinetobacter* are the only GNB associated with consistently increasing proportions of HAIs. *Acinetobacter baumannii* is predicted to become a predominant cause of HAIs due to the selective pressure from antimicrobials in intensive care units (ICUs), in addition to its incredible ability to acquire resistance.¹

In the hospital setting, carbapenems, such as imipenem, are reserved for the treatment of the most severely ill patients due to their high potency, broad-spectrum activity, and good safety profile.² In this study, the prevalence of Gram-negative rods causing HAIs was investigated in three Egyptian ICUs. *A. baumannii* showed the highest percentages of carbapenem resistance. The

mechanism of resistance and clonal relatedness were further studied.

2. Methods

A total of 547 HAIs in three ICUs were investigated in the period between January 2011 and September 2012. The three hospitals were: hospital A, 6th October Hospital (13 ICU beds); hospital B, MUST Hospital (seven ICU beds); and hospital C, National Cancer Institute (12 ICU beds). Only cases confirmed to be HAIs (i.e., infection was first present on or after the third hospital day) were included.

Antimicrobial susceptibility and minimum inhibitory concentration (MIC) testing were done using standard protocols.³ Evaluation of the molecular mechanisms of carbapenem resistance in the carbapenem-resistant *A. baumannii* (CRAB) isolates was performed using standard assays, such as the modified Hodge test, inhibitor-potentiated disk diffusion (IPD), effect of the efflux pump inhibitor carbonyl cyanide chlorophenylhydrazone (CCCP), and PCR detection of *bla*_{OXA-51}-like, *bla*_{OXA-23}-like, and class 1 integrase (*int1*) genes, as reviewed by Durante-Mangoni and Zarrilli.⁴ Finally,

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clonal relatedness between the isolates was investigated by enterobacterial repetitive intergenic consensus (ERIC)-PCR.

3. Results and discussion

A total of 547 non-duplicate GNB were recovered from the three hospitals. The majority of infections were due to *Klebsiella spp*; the frequencies of isolation of the different species and types of infections associated with each species are summarized in Table 1.

A. baumannii showed the highest imipenem resistance (74%). With the exception of two recent studies,^{5,6} almost nothing is known about *A. baumannii* resistance patterns in Egypt. For this reason, all 39 CRAB isolates identified in this study were subjected to further investigation. The former study (Al-Hassan et al.⁵) included isolates from pediatric patients obtained from hospital C, however the average age of our cases was 48.6 years.

Antimicrobial susceptibility testing showed that 100% of the CRAB isolates were susceptible to colistin. However, the resistance

Table 1
 Distribution of bacterial species causing different types of infections included in the study

	UTI, n (%)	RTI, n (%)	Wound infections, n (%)	Bacteremia, n (%)	Meningitis, n (%)	Total, n (%)
<i>Acinetobacter baumannii</i>	6 (11)	24 (45)	22 (42)	1 (2)	-	53 (10)
<i>Escherichia coli</i>	36 (35)	19 (18)	44 (43)	2 (2)	2 (2)	103 (19)
<i>Enterobacter spp</i>	1 (1)	1 (1)	5 (5)	2 (2)	-	9 (1.5)
<i>Klebsiella spp</i>	41 (19)	71 (33)	94 (43)	9 (4)	2 (1)	217 (40)
<i>Proteus spp</i>	10 (67)	-	5 (33)	-	-	15 (2.5)
<i>Pseudomonas spp</i>	24 (16)	37 (25)	86 (57)	1 (1)	2 (1)	150 (27)
Total	118 (21.6)	152 (27.8)	256 (46.8)	15 (2.7)	6 (1.1)	547 (100)

UTI, urinary tract infections; RTI, respiratory tract infections.

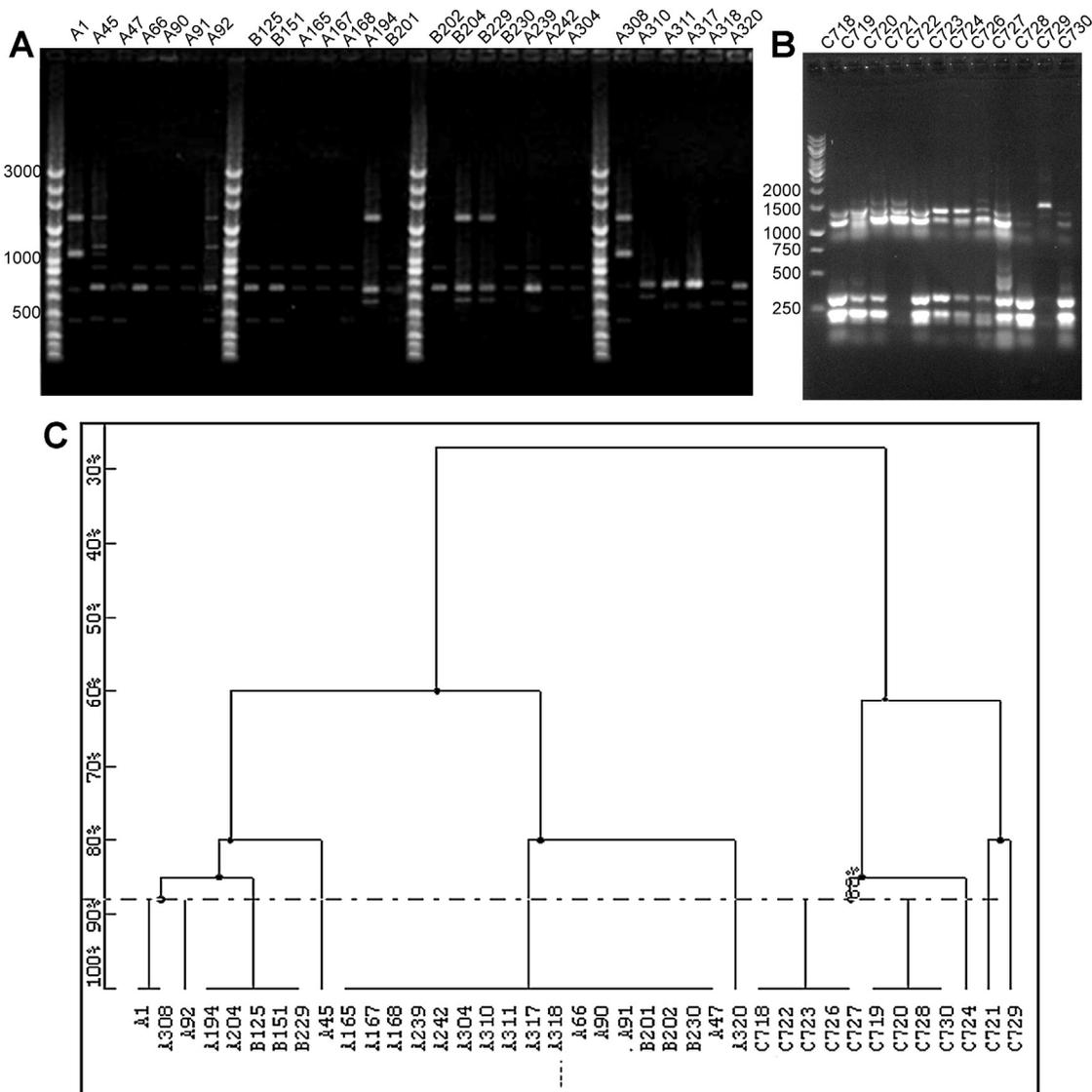


Figure 1. DNA agarose gel showing the products of the ERIC-PCR on the *Acinetobacter baumannii* carbapenem-resistant isolates from hospitals A and B (A), and hospital C (B). (C) Dendrogram representing genetic relationships between *A. baumannii* isolates based on ERIC-PCR fingerprints. The isolates are labeled according to the source hospital, i.e., for isolates obtained from hospital A, the label starts with the letter A, etc.

level to other antibiotics was 100% for each of meropenem, ceftazidime, cefepime, ciprofloxacin, and piperacillin/tazobactam, and 80% or more to amikacin, aztreonam, gentamicin, and tobramycin. The MIC values for imipenem were over 16 µg/ml for 97.4% of the isolates, and 53.8% and 33.3% of the isolates had values greater than 512 µg/ml for amikacin and gentamicin, respectively. In accordance with previous findings, the respiratory tract is the main site of infection and colistin is considered almost the only treatment choice left for the management of *A. baumannii* infections.

Phenotypic assays indicated that 82% of the CRAB isolates had carbapenemase activity; 2.5% had metallo-beta-lactamase (MBL) activity, and in no isolate did the overexpression of proton gradient-dependent efflux pumps contribute to the observed resistance.

Multiplex PCR showed the presence of the *bla*_{OXA-51}-like and the *bla*_{OXA-23}-like genes in all of the CRAB isolates. The *bla*_{VIM} gene was detected only in the isolate with MBL activity. OXA-23-type carbapenemase-producing *A. baumannii* are becoming increasingly widespread, with reports emerging from Europe, the USA, Australia, Asia, Africa, and the Middle East.⁷

Integrase gene detection is considered a good marker for the dissemination of epidemic *Acinetobacter* strains and it can be responsible for the integration of resistance markers either in the chromosome or plasmids.⁸ The *int1* gene was detected in 85% of our isolates, and PCR analysis indicated that *bla*_{OXA-23} was carried on a plasmid in 28.2% of the CRAB isolates.

ERIC-PCR analysis revealed that 16 isolates – 13 from hospital A and three from hospital B – showed identical patterns, however those isolated from hospital C were unrelated to them, although they showed closely related patterns amongst themselves (Figure 1). This result indicates that clonal expansion of certain CRAB is taking place in some of the Egyptian hospitals. PCR-based fingerprinting methods such as ERIC-PCR are simple, rapid, and low-cost methods with high discrimination for the identification and differentiation of *Acinetobacter* spp.⁹ It has been concluded that both pulsed field gel electrophoresis (PFGE) and PCR-based fingerprinting are useful for typing *Acinetobacter calcoaceticus*-

baumannii. However, PFGE can detect minor mutations in an outbreak strain.¹⁰

Our results indicate that the observed imipenem resistance is due to the spread of OXA-23-producing clones. The clinical significance of these isolates is of great concern as it emphasizes the importance of having effective infection control measures in Egyptian hospitals; strict adherence to these measures is required to prevent the spread of these resistant organisms.

Ethics statement: All experiments performed in this study were approved by the Research Ethics Committee of the Faculty of Pharmacy, Cairo University.

Conflict of interest: The authors declare no conflict of interest.

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