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ORIGINAL ARTICLE

# Clonal dissemination of extensively drug-resistant *Acinetobacter baumannii* producing an OXA-23 β-lactamase at a teaching hospital in Shanghai, China

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**KEYWORDS**
*Acinetobacter baumannii*;
Clonal dissemination;
Extensively drug-resistant;
Risk factor

*Background/Purpose:* Extensively drug-resistant (XDR) *Acinetobacter baumannii* presents a serious therapeutic and infection control challenge. This study aimed to explore the causes for the rapid increase of XDR *A. baumannii* at a teaching hospital in Shanghai.
*Methods:* All consecutive clinical isolates of XDR *A. baumannii* were collected from January to December 2010 at Huashan Hospital in Shanghai. The prevalence of carbapenemase genes was investigated by polymerase chain reaction (PCR) amplification. Genetic relatedness of the isolates was determined by enterobacterial repetitive intergenic consensus-PCR and multilocus sequence typing. A retrospective case–control study was performed for the identification of risk factors of XDR *A. baumannii* infections.
*Results:* All 106 XDR *A. baumannii* isolates carried the $bla_{OxA-23}$ gene and were resistant to all antimicrobial agents tested, except colistin, tigecycline and cefoperazone-sulbactam. One hundred and five of the strains belonged to clonal complex 92 by multilocus sequence typing, and 78 were classified as clone A1 by enterobacterial repetitive intergenic consensus-PCR. Intensive care unit residency at the time of isolation, recent general anesthesia, the number of previous antibiotic classes administered and previous hospitalization were identified as risk factors by case-control study. Efficacy rates were 62.5% (5/8), 47.4% (9/19), and 42.9% (3/7) when the XDR patients were treated with cefoperazone–sulbactam, carbapenems, or both cefoperazone–sulbactam and carbapenem, alone or in combination with other agents, respectively.

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*Conclusion:* XDR *A. baumannii* producing OXA-23 β-lactamase was clonally disseminated at a university hospital in Shanghai. Cefoperazone—sulbactam and carbapenems alone or combined with other antibiotics may benefit XDR *A. baumannii* infections in the absence of other effective antibiotics.
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## Introduction

*Acinetobacter baumannii* is notorious for its remarkable ability to acquire antibiotic resistance and cause persistent nosocomial infections.[1,2] The propensity of *A. baumannii* to be multidrug-resistant (MDR) or extensively drug-resistant (XDR) presents therapeutic and infection control challenges. The problem is especially acute in China where polymyxins remain unavailable and tigecycline only became available in 2011. Invasive procedures such as mechanical ventilation, intensive care unit (ICU) stay, recent surgery, and use of broad-spectrum antibiotics are reported as risk factors for colonization or infection by MDR *A. baumannii*.[3] Due to the wide application of broad-spectrum antibiotics, the resistance rates of *A. baumannii* to most classes of antibiotics have continually increased during the past decades.

The emergence of carbapenem-resistant *A. baumannii* (CRAB) has been described as the sentinel event of clinically relevant antimicrobial resistance. The MYSTIC surveillance program in 2008 demonstrated that 57.4% of *A. baumannii* isolates were resistant to meropenem and 47.9% resistant to imipenem in Europe.[4] Among CRAB isolates, the production of acquired carbapenem hydrolyzing class D β-lactamases, including those belonging to the OXA-23, -24, and -58 families, and overproduction of intrinsic class D β-lactamase OXA-51 family are the most prevalent mechanisms for carbapenem resistance.[2] In China, surveillance by the CHINET project showed that imipenem resistance rate of *A. baumannii* increased from 30.1% in 2006 to 57.1% in 2010.[5,6] Mirroring this national trend, the imipenem resistance rate of *A. baumannii* at our hospital increased from 29.9% to 62.7% during the same period. In addition, the prevalence of XDR *A. baumannii* increased at our hospital from 3.7% in 2008 to 19.6% in 2009 and to 41.9% in 2010. Few clinical data are available on XDR *A. baumannii* from China.

In order to explore the causes for the rapid increase of XDR *A. baumannii* and to identify whether a nosocomial dissemination of XDR *A. baumannii* with molecular relatedness was occurring at our hospital, the clonality of the isolates and the presence of carbapenemase genes were determined. Furthermore, the risk factors for hospital-acquired XDR *A. baumannii* were analyzed by a case—control study and antimicrobial treatment options were evaluated.

## Materials and methods

### Hospital setting and bacterial isolates

This study was conducted at the Huashan Hospital, a tertiary care university hospital, in Shanghai, China, which has approximately 1300 beds, including 260 neurosurgical beds. All consecutive and nonduplicate clinical XDR *A. baumannii* isolates ($n = 106$) were collected at the hospital from January to December 2010. Rapid species identification was performed by one-tube multiplex polymerase chain reaction (PCR).[7] *A. baumannii* was identified if two PCR products were yielded: a 425-bp internal control amplicon corresponding to the *recA* gene of *Acinetobacter* spp. and the 208-bp fragment of the 16S—23S rRNA intergenic spacer region of *A. baumannii*. Non-*baumannii Acinetobacter* isolates, which yielded the 425-bp PCR product alone, were excluded in this study. Isolates were recovered from the respiratory tract ($n = 76$), drainage fluid ($n = 9$), urine ($n = 6$), wound ($n = 6$), blood ($n = 5$), catheter ($n = 3$) and cerebrospinal fluid ($n = 1$).

The criteria set by the National Healthcare Safety Network were used to determine whether isolation of *A. baumannii* indicated colonization or infection.[8] Hospital acquisition was defined as detection of colonization or infection at least 72 hours after arrival at the medical facility. XDR strains first isolated from patients admitted to hospital within 72 hours or patients who had *A. baumannii* infections in other hospitals prior to this admission were defined as imported strains.

### Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) of 16 antimicrobial agents were measured by agar dilution according to the recommendations of the Clinical and Laboratory Standards Institute 2014.[9] Tigecycline MICs were determined by broth microdilution using Food and Drug Administration clinical MIC breakpoints for *Enterobacteriaceae*: $\leq$2 mg/L for susceptible and $\geq$8 mg/L for resistant. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains.

CRAB was defined if an *A. baumannii* isolate was resistant to both imipenem and meropenem. MDR *A. baumannii* is defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories. XDR *A. baumannii* is defined by nonsusceptible to at least one agent in all but two or fewer antimicrobial categories (remains susceptible to only 1 or 2 categories).[10]

### Molecular typing

Molecular typing of isolates was performed by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and multilocus sequence typing (MLST). Primer ERIC-2 was used for amplification. PCR conditions were described previously.[11] The DNA banding patterns were analyzed using

Quantity One software. ERIC-PCR DNA patterns were compared and interpreted according to the number of band differences.[12] MLST was carried out as described previously. In brief, internal fragments of seven housekeeping genes (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*) were PCR amplified, purified, and sequenced.[13] A clonal complex (CC) comprised a founding sequence type (ST) as a common ancestor and other closely related STs descended from the predicted founding genotype, defined by adjoining single locus variants.

### Detection of carbapenemase genes

Genes coding for class A, B, and D carbapenemases were investigated by PCR. Multiplex PCR was performed with primers that anneal to $bla_{\text{OxA-23}}$-like, $bla_{\text{OxA-24}}$-like, $bla_{\text{OxA-51}}$-like, and $bla_{\text{OxA-58}}$-like carbapenemases.[2] The entire $bla_{\text{OxA-23}}$-like and $bla_{\text{OxA-51}}$-like coding regions were amplified and sequenced using primer pairs as previously described.[14,15] Metallo-β-lactamase, $bla_{\text{NDM}}$, was detected with PCR conditions and primers described previously.[16] To detect the presence of $bla_{\text{KPC}}$, $bla_{\text{IMP}}$ and $bla_{\text{VIM}}$, the following primer sets were used: KPC-F, GCTCAGGCGC AACTGTAAGT and KPC-R, GTCCAGACGGAACGTGGTAT; IMP-F, GGAATAGAGTGGCTTAATTCTC and IMP-R, GCAGCCAAAC CACTAWGTTATCT; VIM-F, GCACTTCTCGCGGAGATTG and VIM-R, ATCGAATGCGCAGCACC. Reaction conditions of PCR were 94°C for 5 minutes and 35 cycles of 94°C for 30 seconds, 53°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes.

### Case—control study and chart review

The case group included patients infected or colonized with hospital-acquired XDR *A. baumannii*. The control group included all patients with hospital-acquired non-XDR *A. baumannii* at main campus of Huashan Hospital in 2010 were included. The medical records of patients in the case and control groups were reviewed in order to extract epidemiologic data and clinical information. Prior antibiotic exposures were defined as at least 24 hours of therapy during the 14 days prior to isolation of the organism for the *A. baumannii*-positive patients. Records were examined to identify isolation of other MDR organisms, such as *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus* spp. and methicillin-resistant *Staphylococcus aureus*. In-hospital mortality was recorded; death was attributed to infection when it occurred during the acute phase of the infection or when the patient was still receiving treatment. Time at risk was defined as length of stay prior to *A. baumannii* isolation for *A. baumannii*-positive patients.

Therapy of XDR *A. baumannii* infections was also evaluated. Response to treatment was defined as normalization of vital signs and resolution of clinical symptoms associated with infection (resolution of fever), improvement in arterial blood-gas values, normal blood counts, radiological improvement, and negative culture from the source related to infection. Efficacy rates of cefoperazone—sulbactam, carbapenems, and aminoglycoside alone or combined with other antibiotics were calculated and compared. The duration of treatment varied from 5 days to 3 weeks. The clinical response was evaluated 3 days after initiating treatment.

The study was approved by the Institutional Review Board of the Huashan Hospital, Shanghai, China (KY2013-314 and KY2013-316).

### Statistical analysis

Continuous variables were presented as mean ± standard deviation, and comparative analysis was conducted using an independent sample nonparametric test. A Chi-square test and Fisher's exact test were used for comparative analysis of categorical variables, and the odds ratio was calculated with 95% confidence intervals. To determine independent risk factors, multivariate analysis was performed using forward stepwise logistic regression with $\alpha$ to enter equaling 0.05 and $\alpha$ to remove equaling 0.10. Statistical significance was defined as $p < 0.05$. SPSS 20.0 (SSPS Inc., Chicago, IL, USA) was used for all analyses.

## Results

All 106 *A. baumannii* isolates were resistant to every antimicrobial agent tested except that 105 strains (99.1%) were susceptible to colistin, 55 (51.9%) susceptible to tigecycline and three (2.8%) susceptible to cefoperazone—sulbactam. One strain had a colistin MIC of 256 mg/L (Table 1).

All strains had more than 10 bands for the ERIC profile. Twelve distinct clonal types were identified by ERIC-PCR with clone A1 accounting for 73.6% (78/106; Table 2). Eight distinct STs were identified by MLST (Fig. 1A). Seven STs of ST138, ST395, ST75, ST92, ST90, ST118 and ST735 accounted for 99.1% (105/106) and were clustered into clonal complex 92. Although ST138 was the most common ST comprised 30.2% (32/106) in this study, the six STs were single-locus variants of ST92, different from each other only in the *gpi* locus (Table 2). ST92, a globally distributed type, was the predicted founder of CC92 in *A. baumannii* MLST database (Fig. 1B). Only one non-CC92 STs (ST671) strain identified in the present study fell into distinct singleton (Table 2). Of 78 ERIC-PCR A1 strains, there were 23 each of ST138 and ST395, 17 ST92, and 15 ST75, all belonging to CC92.

OXA-type carbapenemase including $bla_{\text{OxA-23}}$ and $bla_{\text{OxA-66}}$ genes were present in all 105 isolates belonging to clonal complex 92. The presence of $bla_{\text{OxA-23}}$ and $bla_{\text{OxA-69}}$ was revealed in the strain belonged to ST671. No $bla_{\text{KPC}}$, $bla_{\text{NDM}}$, $bla_{\text{VIM}}$, $bla_{\text{IMP}}$, $bla_{\text{OxA-24-like}}$ and $bla_{\text{OxA-58-like}}$ genes were detected.

Seven outpatients and six inpatients from whom complete data could not be obtained were omitted from further analysis. Of the remaining 93 cases, 81 (87.1%) were isolated from patients with hospital-acquired infections who had prolonged hospital stays (average, 68.6 days), and 12 (12.9%) were defined as imported strains. Among 93 patients, 41 (44.1%) received neurosurgery and 38 (40.9%) were admitted to the ICU. Seventy-two patients (77.4%) had a tracheostomy or endotracheal tube, and 51 patients (55.4%) received mechanical ventilation. The overall in-hospital mortality was 17.2% (16/93).

**Table 1** Antimicrobial susceptibility of 106 extensively drug-resistant *Acinetobacter baumannii* isolates (mg/L)

| Antimicrobial | MIC range | $MIC_{50}$ | $MIC_{90}$ | Susceptibility percentage |
|---|---|---|---|---|
| Piperacillin | 128–512 | 512 | 512 | 0 |
| Ceftazidime | 32–256 | 64 | 256 | 0 |
| Cefepime | 32–256 | 64 | 128 | 0 |
| Piperacillin–tazobactam | 128–256 | 256 | 256 | 0 |
| Ampicillin–sulbactam | 32–128 | 32 | 64 | 0 |
| Cefoperazone–sulbactam[a] | 16–128 | 32 | 64 | 2.8% |
| Imipenem | 16–256 | 32 | 64 | 0 |
| Meropenem | 16–128 | 16 | 64 | 0 |
| Minocycline | 8–32 | 8 | 16 | 0 |
| Tigecycline[b] | 1–16 | 2 | 8 | 51.9% |
| Ciprofloxacin | 8–128 | 64 | 64 | 0 |
| Amikacin | 64–256 | 256 | 256 | 0 |
| Trimethoprim –sulfamethoxazole | 8–16 | 16 | 16 | 0 |
| Colistin | 0.25–256 | 0.5 | 0.5 | 99.1% |
| Rifampicin | 2–16 | 4 | 8 | —[c] |
| Sulbactam | 8–256 | 32 | 32 | —[c] |

[a] Cefoperazone-sulbactam MICs of ≤16/8 mg/L, 32/16 mg/L and ≥64/32 mg/L were interpreted as susceptible, intermediate and resistant, respectively.
[b] Tigecycline MICs of ≤2 mg/L and ≥8 mg/L were interpreted as susceptible and resistant.
[c] No Clinical and Laboratory Standards Institute breakpoints.
MIC = minimal inhibitory concentration.

Of the 93 patients, 43 were classified as infected and 50 as colonized with XDR *A. baumannii*. Among the 43 infected patients, 29 had hospital-acquired pneumonia (including 13 ventilator-associated pneumonia), four each bloodstream infections and abdominal infections, three urinary tract infections, two wound infections, and one meningitis. No statistically significant difference was observed between infected and colonized patients in terms of clinical characteristics, except that colonized patients had a longer time at risk (colonized patients, average 43.7 days vs. infected patients, 15.6 days, $p = 0.04$), a longer duration of hospital stay (average 100.8 vs. 31.0 days, $p = 0.002$),

**Table 2** Multilocus sequence typing (MLST) and enterobacterial repetitive intergenic consensus (ERIC) typing of 106 extensively drug-resistant *Acinetobacter baumannii* isolates

| MLST type | | No. of isolates | ERIC type | No. of different bands compared with ERIC A1 | Genetic relationship with ERIC A1[b] |
|---|---|---|---|---|---|
| ST[a] | *gpi* allele | | | | |
| 75 | 11 | 15 | A1 | 0 | — |
| 75 | 11 | 4 | G | 2 | Closely related |
| 75 | 11 | 2 | E | 3 | Closely related |
| 75 | 11 | 1 | D | 2 | Closely related |
| 92 | 7 | 17 | A1 | 0 | — |
| 92 | 7 | 1 | H | 2 | Closely related |
| 92 | 7 | 1 | J | 2 | Closely related |
| 138 | 50 | 23 | A1 | 0 | — |
| 138 | 50 | 2 | A2 | 1 | Different subtype |
| 138 | 50 | 1 | H | 2 | Closely related |
| 138 | 50 | 6 | K | 2 | Closely related |
| 395 | 58 | 23 | A1 | 0 | — |
| 395 | 58 | 1 | L | 4 | Possibly related |
| 90 | 62 | 1 | B | 3 | Closely related |
| 90 | 62 | 5 | F | 3 | Closely related |
| 735 | 197 | 1 | E | 3 | Closely related |
| 118 | 3 | 1 | I | 2 | Closely related |
| 671 | — | 1 | C | 7 | Unrelated |

[a] All the STs except ST671 belong to CC92, different from each other only in the *gpi* locus.
[b] Genetic relationship was identified by the number(s) of different ERIC-PCR bands compared with ERIC A1: 1 band, different subtype; 2–3 bands, closely related; 4–6 bands, possibly related; ≥7 bands, unrelated.

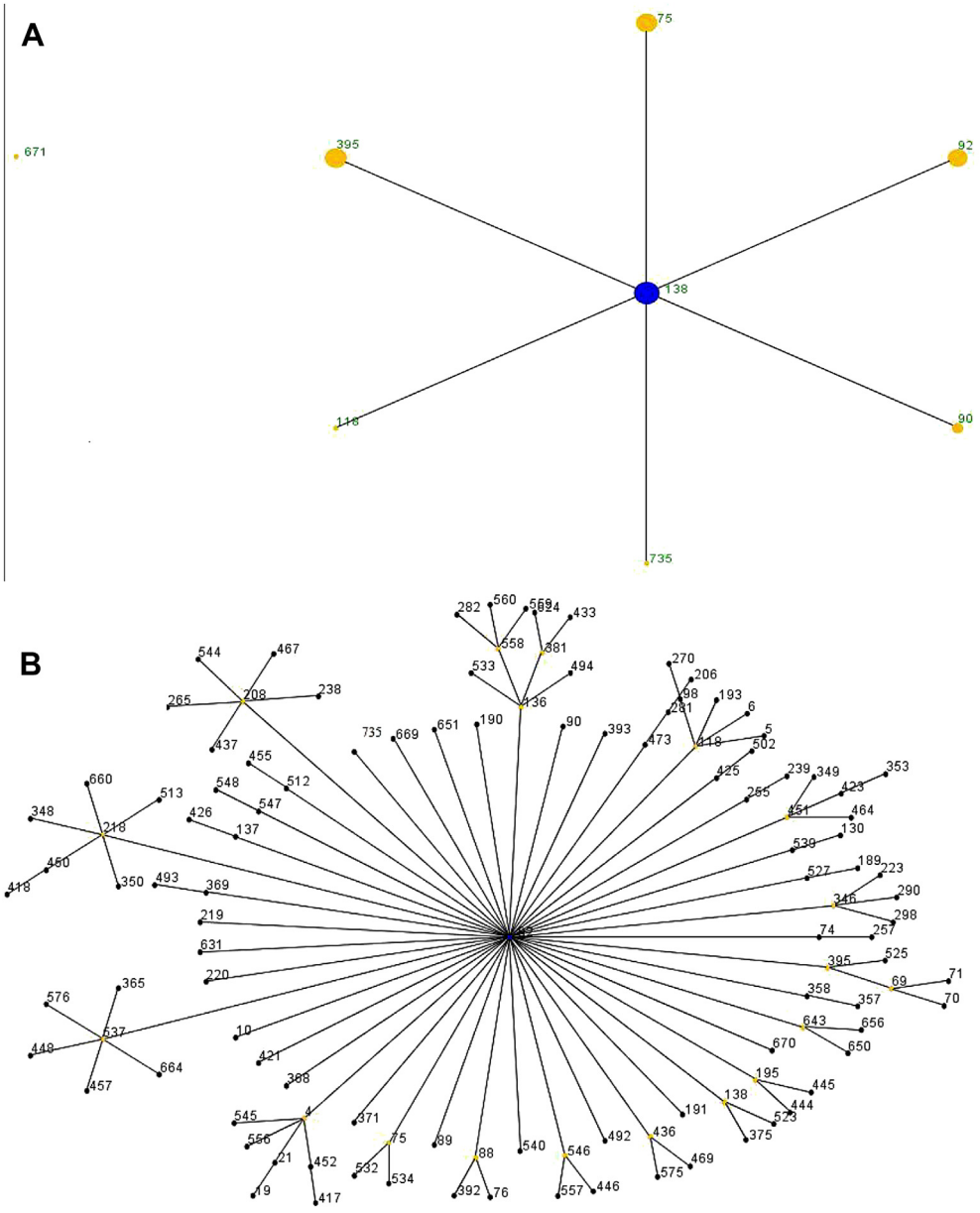


**Figure 1.** (A) The relatedness of the sequence types (STs) of 106 strains in this study. (B) eBURST population snapshot of clonal complex 92 (CC92) from the *A. baumannii* MLST website. eBURST was performed using six as the minimum identical loci for the definition of CC and three as the minimum single locus variants. The radial diagram reflects the predicted evolutionary descent from the founder ST. The size of the circle corresponds to the number of isolates belonging to a ST.

and a higher frequency of isolation of other MDR bacteria (96.0% vs. 58.1%, $p < 0.001$; Supplementary Table).

The 13 patients with incomplete clinical data, 12 patients with imported XDR *A. baumannii*, and four patients with hospital stay <48 hours could not be evaluated and were not included in further analysis. The case group consisted of 77 patients, and control group with hospital-acquired non-XDR *A. baumannii* included 212 patients (Table 3). There was no difference in the sex and mean age. The isolates were mostly recovered from the respiratory tract both in the case (71.4%, 55/77) and control group (70.8%, 150/212). More patients (40.3%) in the case group were in an ICU at the time of *A. baumannii* isolation and had more previous hospitalization (72.7%). The presence of gastric tube, bladder catheter, intubation or tracheostomy, intravascular catheter, and history of surgery were also associated with acquisition of XDR *A. baumannii*. Patients in the case group were previously treated with more classes of antibiotics (4.2 classes), including carbapenems and β-lactam/β-lactamase combinations. However, a difference in the underlying diseases was not significant by univariate analysis, including cardiovascular disease, cerebral vascular accident, trauma, tumor, and diabetes. Four independent risk factors were identified in the multivariate analysis: location in an ICU, recent general anesthesia, number of previous antibiotic classes administered, and previous hospitalization (Table 4).

**Table 3** Comparison of clinical characteristics between patients with extensively drug-resistant (XDR) and non-XDR *Acinetobacter baumannii* isolates

| Clinical characteristics | Case (XDR isolates, $n = 77$) | Control (non-XDR *A. baumannii*, $n = 212$) | $p$ | Odds ratio (95% CI) |
|---|---|---|---|---|
| Male sex, *n* (%) | 52 (67.5%) | 147 (69.3%) | 0.769 | 0.92 (0.53—1.61) |
| Age, mean y (range) | 59.5 (10—93) | 62.7 (17—98) | 0.140 | 0.99 (0.98—1.00) |
| In ICU at the time of isolation, *n* (%) | 31 (40.3%) | 24 (11.3%) | <0.001 | 5.28 (2.83—9.84) |
| Neurosurgery patients, *n* (%) | 29 (37.7%) | 73 (34.4%) | 0.767 | 1.08 (0.64—1.84) |
| Time at risk, mean d (range) | 30.0 (4—350) | 25.9 (4—613) | 0.119 | 1.00 (1.00—1.01) |
| Predisposing factors, *n* (%) | | | | |
| Gastric tube | 67 (87.0%) | 159 (75.0%) | 0.029 | 2.23 (1.07—4.65) |
| Proton pump inhibitors | 65 (84.4%) | 170 (80.2%) | 0.415 | 1.34 (0.66—2.70) |
| Bladder catheter | 62 (80.5%) | 138 (65.1%) | 0.012 | 2.22 (1.18—4.16) |
| Intubation/tracheostomy | 58 (75.3%) | 109 (51.4%) | <0.001 | 2.88 (1.61—5.17) |
| Suctioning/bronchoscopy | 55 (71.4%) | 129 (60.8%) | 0.098 | 1.72 (0.97—3.04) |
| Intravascular catheter | 52 (67.5%) | 111 (52.4%) | 0.020 | 1.89 (1.09—3.27) |
| Surgery | 45 (58.4%) | 111 (52.4%) | 0.033 | 1.28 (0.76—2.17) |
| General anesthesia | 38 (49.4%) | 92 (43.4%) | 0.368 | 1.27 (0.75—2.14) |
| Glucocorticoid therapy | 26 (33.8%) | 71 (33.5%) | 0.965 | 1.01 (0.58—1.76) |
| Previous use of antibiotics, *n* (%) | | | | |
| Carbapenems | 48 (62.3%) | 91 (42.9%) | 0.003 | 2.20 (1.29—3.76) |
| β-lactam/β-lactamase combinations | 43 (55.8%) | 85 (40.1%) | 0.017 | 1.87 (1.11—3.18) |
| Fluoroquinolones | 24 (31.2%) | 51 (24.1%) | 0.220 | 1.42 (0.80—2.53) |
| Number of previous antibiotic classes used | 4.2 (0—14) | 2.8 (0—14) | <0.001 | 1.21 (1.10—1.33) |
| Previous hospitalization within 1 mo, *n* (%) | 56 (72.7%) | 113 (53.3%) | 0.003 | 2.34 (1.32—4.13) |
| Isolation of other MDR bacteria, *n* (%) | 59 (76.6%) | 156 (73.6%) | 0.601 | 1.18 (0.64—2.16) |
| Duration of hospital stay, mean d (range) | 67.8 (3—734) | 84.4 (4—1613) | 0.600 | 1.00 (1.00—1.00) |
| Overall in-hospital mortality, *n* (%) | 16 (20.8%) | 31 (14.6%) | 0.210 | 1.53 (0.78—2.99) |

CI = confidence interval; ICU = intensive care unit; MDR = multidrug-resistant.

Of 43 XDR *A. baumannii* infected patients, eight were treated with intravenous cefoperazone—sulbactam alone or combined with other antibiotics (except carbapenems), and were effective in five patients (62.5%). Both cefoperazone—sulbactam-susceptible *A. baumannii*-infected patients were evaluated as effective (the third cefoperazone—sulbactam-susceptible isolate was evaluated as colonization). Cefoperazone—sulbactam (2:1) was given in dosages ranging from 3.0 g 12-hourly to 6-hourly. Intravenous carbapenems alone or combined with other antibiotics (except cefoperazone—sulbactam) were used in 19 patients, and were effective in 9 (47.4%) of these patients as assessed by clinical and laboratory improvement. The most frequently used carbapenem was meropenem (16/19), which was given in dosages ranging from 1.0 g 6—12-hourly to 2.0 g 8-hourly. Other carbapenems used were panipenem and imipenem. Seven patients were given both carbapenem and cefoperazone—sulbactam (3 were combined with doxycycline or minocycline), which was effective in three (42.9%) patients (Table 5).

## Discussion

The present study revealed that the rapid increase of XDR *A. baumannii* at our hospital between January to December 2010, was due to clonal dissemination of closely related OXA-23-producing organisms. Both ERIC-PCR and MLST showed that almost all isolates were clonally related and CC92 was responsible for the spread. On a global scale, ST92 is the predicted founder of CC92, the largest and most geographically diverse clonal complex by MLST.[17] Our results revealed that our facility in Shanghai, China is included in this global epidemic. So far, ST92 isolates have been reported from Spain, Korea, Hong Kong, Australia and America according to the *A. baumannii* MLST web site

**Table 4** Multivariate analysis of risk factors related to extensively drug-resistant (XDR) *Acinetobacter baumannii* isolation (against non-XDR *A. baumannii*)

| Variables | β | Odds ratio (95% CI) | $p$ |
|---|---|---|---|
| In ICU at the time of isolation | 1.794 | 6.016 (1.10—2.49) | <0.001 |
| General anesthesia | 0.868 | 2.382 (0.24—1.50) | 0.007 |
| Number of previous antibiotic classes used | 0.194 | 1.215 (0.08—0.30) | <0.001 |
| Previous hospitalization within 1 mo | 0.632 | 1.882 (0.01—1.25) | 0.045 |

CI = confidence interval.

**Table 5** Antimicrobial therapy and treatment efficacy rate of 43 extensively drug-resistant (XDR) *Acinetobacter baumannii* infections

| Antimicrobial | Combined with | Total (*n*) | Effective (*n*) | Ineffective (*n*) | Efficacy rate (%) |
|---|---|---|---|---|---|
| Carbapenems | Alone | 12 | 6 | 6 | 50% |
| | Isepamicin | 1 | 1 | 0 | 100% |
| | Doxycycline/ minocycline | 6 | 2 | 4 | 33.3% |
| Cefoperazone—sulbactam | Alone | 4 | 3 | 1 | 75% |
| | Amikacin/isepamicin | 2 | 2 | 0 | 100% |
| | Doxycycline/ minocycline | 2 | 0 | 2 | 0% |
| Carbapenems + Cefoperazone − sulbactam | Alone | 4 | 2 | 2 | 50% |
| | Doxycycline/ minocycline | 3 | 1 | 2 | 33.3% |
| Aminoglycosides | Amikacin alone | 1 | 0 | 1 | 0% |
| Isepamicin | Doxycycline/ minocycline | 1 | 1 | 0 | 100% |
| No antimicrobial treatment | | 7 | 2 | 5 | 28.6% |

(http://pubmlst.org/abaumannii/). We also observed that, in our highly clonal isolate collection, ERIC-PCR was able to cluster CC92 isolates accurately. ERIC-PCR is a very inexpensive typing method compared with MLST or even pulsed-field gel electrophoresis. Our findings suggest that ERIC-PCR, complemented by selective use of MLST, may be a cost-efficient approach in addressing hospital epidemiology of *A. baumannii*.

Carbapenemase-producing strains of *A. baumannii* have been involved in outbreaks in Europe, Asia, and both North and South America.[2,18–20] The carbapenemases produced by such strains are mostly class D oxacillinases,[2,18,20] although metalloenzymes of the IMP, VIM, and NDM families have also been detected in some well-defined geographical regions.[2] All XDR isolates in our clonal dissemination harbored $bla_{OxA-23}$ and $bla_{OxA-66}$ (but one $bla_{OxA-69}$) genes, consistent with previous reports.[2,18,20]

Although several risk factors have been previously associated with MDR/CRAB colonization and infection,[3,21,22] to date there has been little research on the risk factors for XDR *A. baumannii*. One study from Taiwan identified prior use of imipenem, meropenem, piperacillin/tazobactam, or fourth-generation cephalosporins and >30 days bed-ridden as independent risk factors for XDR *A. baumannii* hospital-acquired infection.[23] We identified four risk factors for isolation of XDR *A. baumannii* in our retrospective case—control study. Among them, general anesthesia has not been reported as a risk factor in previous studies. In our study, 41 (44.1%) patients were neurosurgery patients, and most of them had received general anesthesia during surgery. General anesthesia is known to increase the risk of pulmonary infections in general.[24] The risk related to the number of antibiotics presumably reflects easier selection of resistant strains, consistent with previous studies.[3,21,25,26]

ICU location was associated with XDR *A. baumannii* colonization and infection in this study, which was consistent with previous reports.[3,21,22] Previous hospitalization increased the probability of XDR *A. baumannii* isolation. Huashan Hospital is a leading referral hospital for eastern China, and so collects patients and their resistant flora from a broad population, which probably contributed in making this a significant risk factor.

The neurosurgery department utilizes 260 of the 1326 beds (19.6%) at our hospital. Our results indicate that neurosurgery patients accounted for 44.1% of those with XDR *A. baumannii*. However, in our case—control study, such patients showed no significant difference between the case and control group, implying that both XDR and non-XDR *A. baumannii* were common in neurosurgical patient.

Cefoperazone—sulbactam is a commonly used antimicrobial for the treatment of CRAB or XDR *A. baumannii* infections in China because of the lack of polymyxins and tigecycline (prior to 2011). National bacterial surveillance project CHINET showed that cefoperazone-sulbactam and minocycline had the lowest resistance rates of both 31% (in addition, 25% were intermediate for cefoperazone—sulbactam) followed by ampicillin—sulbactam (53% resistance) for 5523 strains of *Acinetobacter* spp., of which 90% were *A. baumannii* in 2010.[7] In our retrospective analysis, eight of 43 XDR *A. baumannii* patients were treated with cefoperazone—sulbactam alone or in combination with a resulting efficacy rate of 62.5%, which was higher than that of carbapenems alone or in combination (47.4%). This study indicated that high-dose intravenous cefoperazone—sulbactam and carbapenem alone or combined with other antibiotics was effective in some patients and could be considered choices for treatment when other options are not available.

In summary, OXA-23-producing XDR *A. baumannii* disseminated clonally at our referral hospital. Further investigation revealed the role of neurosurgery and general anesthesia in the spread XDR *A. baumannii*. Infection control measures will need to take into account the risk factors for this emerging problem.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

## Ethics approval

Study procedures were approved by the institutional review board.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.jmii.2014.04.005.

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