References

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High rate of colistin dependence in Acinetobacter baumannii

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Sir,

Colistin is currently considered the last treatment option for MDR Acinetobacter infections. Although the current resistance rates to colistin are relatively low, they are increasing and threaten to become a serious problem worldwide.1 In this study, we report the in vitro development of colistin dependence in many colistin-susceptible Acinetobacter baumannii isolates after exposure to colistin.

Initially, we performed population analysis for some colistin-susceptible isolates collected from two different hospitals, Samsung Medical Center (Seoul, Korea) and Samsung Changwon Hospital (Changwon, Korea). We plated 50 μL of bacterial cell suspension (~10⁹ cfu/mL) and its serial dilutions on Mueller–Hinton agar containing 0, 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 mg/L colistin sulphate. Surviving colonies at 10 mg/L were plated on Mueller–Hinton agar with discs of 10 mg colistin. We found that the bacteria grew only near the disc (Figure 1a), which indicated colistin dependence. Next, we performed colistin susceptibility testing for 170 A. baumannii isolates collected from ICUs of Korean hospitals in 2015 and examined colistin-susceptible isolates for colistin dependence by using population analysis and the colistin disc method.

Among 170 A. baumannii isolates, 21 (12.4%) were resistant to colistin (MIC₉₀ 4 mg/L; MIC₅₀ 1 mg/L). Among 149 colistin-susceptible isolates, surviving colonies on agar with 10 mg/L colistin were observed in 96 isolates (64.4%). In addition, we found that 49 colistin-susceptible A. baumannii isolates (32.9%) developed colistin dependence. These isolates were obtained from sputum (23 isolates, 46.9%), blood (17 isolates, 34.7%), wounds (5 isolates, 10.2%) and other locations. Nearly all of the original isolates that developed colistin dependence after exposure to colistin were resistant to imipenem, meropenem, ciperoxacin, ceftazidime, piperacillin, gentamicin, trimethoprim/subbactam and piperacillin/tazobactam (87.8%–98.0%). Twenty-eight isolates (57.1%) were resistant to ampicillin/subbactam. Tigecycline non-susceptibility was identified in nine isolates (18.4%). However, colistin-dependent mutants were resistant to polymyxins, tetracycline, ciperoxacin and piperacillin/tazobactam, but were susceptible to the others, including carbapenems. The 49 isolates developing colistin dependence showed six STs in MLST using the Oxford scheme:2 ST208 (28 isolates, 57.1%), ST191 (12 isolates, 24.5%), ST368 (6 isolates, 12.2%), ST435 (1 isolate), ST451 (1 isolate) and ST1141 (1 isolate). These STs belong to the global clone 2 and have been identified frequently in Korea. ST208 was identified most frequently among the isolates that developed colistin dependence, although ST191 has recently been identified as the most frequently found carbapenem-resistant A. baumannii clone in Korea.3 The results of PFGE showed that the colistin-dependent mutants originated from their corresponding parental isolates (Figure 1b).

Colistin was administered to 45 patients with A. baumannii infection. Among the A. baumannii isolates obtained from these patients, 18 developed colistin dependence, while the remainder did not. We compared 3 and 7 day treatment failure (defined as a death or persistence of bacteria) in patients with and patients without colistin-dependent strains: 66.7% versus 37.0% (3 day; P=0.051) and 44.4% versus 25.9% (7 day; P=0.197).

Colistin dependence was previously reported in an isolate of A. baumannii–Acinetobacter calcoaceticus complex.4 In that study, only 1 of 19 isolates developed colistin dependence. Partial colistin dependence was recently identified in some LPS-deficient strains with mutations in lpxA, lpxC and lpxD.5 In addition to those studies, our study showed that many colistin-susceptible A. baumannii strains develop colistin dependence after exposure to colistin. Although the clinical implication of colistin dependence remains unclear, the high rate of treatment failure in patients with A. baumannii isolates that develop colistin dependence is notable.
Figure 1. (a) Population analysis results and the development of colistin dependence in colistin-susceptible A. baumannii isolates H09-94 and H09-146. The experiments with the isolates were performed twice. Results of the disc diffusion test indicated that colonies surviving at 0 mg/L colistin had colistin susceptibility, while colonies surviving at 10 mg/L colistin were present near the colistin disc, i.e. they had developed colistin dependence. (b) PFGE patterns of six pairs of colistin-susceptible parental isolates and their colistin-dependent mutants.
The identification of colistin dependence in A. baumannii infections may be important because colistin-dependent mutants show increased susceptibility to several antimicrobial agents, including carbapenems. Although the mechanism through which colistin dependence develops is unknown, this increased antimicrobial susceptibility may be due to LPS deficiency, which results in increased penetration of antimicrobials into the bacterial cell. Thus, fast substitution of antimicrobial agents may be required if colistin treatment is ineffective and colistin dependence is identified.

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**Transparency declarations**

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**References**


**Isolation of mecC MRSA in Australia**

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Sir,

We describe the first known occurrence in Australia of MRSA harbouring the mecC gene, which is a novel mecA homologue that encodes methicillin resistance in staphylococci. Although phenotype typically resistant to methicillin, mecC isolates typically yield negative results for conventional mecA PCR and the PBP2a assay and can therefore hamper diagnosis of MRSA infections. First reported in mecA-negative MRSA isolates from humans and cattle, mecC has since been found in MRSA isolated from livestock, wildlife and companion animals, including domestic cats. Reports of mecC have hitherto been restricted to Europe.

In the present case, the MRSA isolate was identified during the first Australian survey into veterinary antimicrobial resistance, which took place from January 2013 to January 2014. Overall, 1200 staphylococci were collected from clinical veterinary samples submitted to Australian diagnostic laboratories. The mecC isolate was collected in February 2013 from the mandible of a 5-year-old domestic cat located in a semi-urban area of outer Melbourne, Victoria. The isolate was spotted by MALDI-TOF and identification of the species-specific nucle gene in whole-genome-sequenced data. Methicillin susceptibility testing was undertaken using CLSI disc diffusion, broth microdilution and Vitek. Phenotypically methicillin resistant by CLSI cefoxitin disc diffusion, the isolate had an oxacillin MIC and a cefoxitin MIC of 2 and 32 mg/L, respectively. Similarly, by Vitek the isolate was cefoxitin screen positive, but oxacillin susceptible (MIC = 2 mg/L). The observed disparity between cefoxitin and oxacillin susceptibility in this mecC isolate is consistent with previous reports. Although resistant to β-lactams, the isolate was susceptible to all other antimicrobials tested, including ciprofloxacin, marbofloxacin, pradofloxacin, enrofloxacin, tetracycline, chloramphenicol, rifampicin and trimethoprim/sulfamethoxazole. mecA was not detected by PCR and the PBP2a slide agglutination test was negative. Using the Illumina MiSeq whole-genome sequencer, the isolate was characterized as ST425 with an SCC mec element. De novo contigs were blasted against an SCC mec type X1 reference (mecC MRSA LGA251, GenBank accession number FR821779.1), which allowed identification of the mecC type 8 ccr cluster (ccrA1 and ccrB3) genes. The mecC gene had 96% and 100% sequence similarity to the mecC described in Staphylococcus aureus LGA251 and Staphylococcus sciuri GVGS2, respectively. De novo contigs were also used to obtain the spa type and to screen for known virulence genes. The isolate was spa type t6292, which has previously been reported in a mecC ST425 isolate from bulk milk in Somerset, UK. The isolate did not harbour the human immune evasion sak, chp and scn genes, which is consistent with S. aureus originating from an animal reservoir, or the lukF-PV and lukS-PV Panton–Valentine leucocidin-associated genes. The Illumina MiSeq results were confirmed by the Alere Technologies StaphyType DNA microarray assay. The contig sequence containing the SCC mec element from this isolate