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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 (~10^9 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 (MIC_{90}, 4 mg/L; MIC_{50}, 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, ciprofloxacin, ceftazidime, cefepime, gentamicin, trimethoprim/sulbactam and piperacillin/tazobactam (87.8%–98.0%). Twenty-eight isolates (57.1%) were resistant to ampicillin/sulbactam. Tigecycline non-susceptibility was identified in nine isolates (18.4%). However, colistin-dependent mutants were resistant to polymyxins, tetracycline, ciprofloxacin 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.

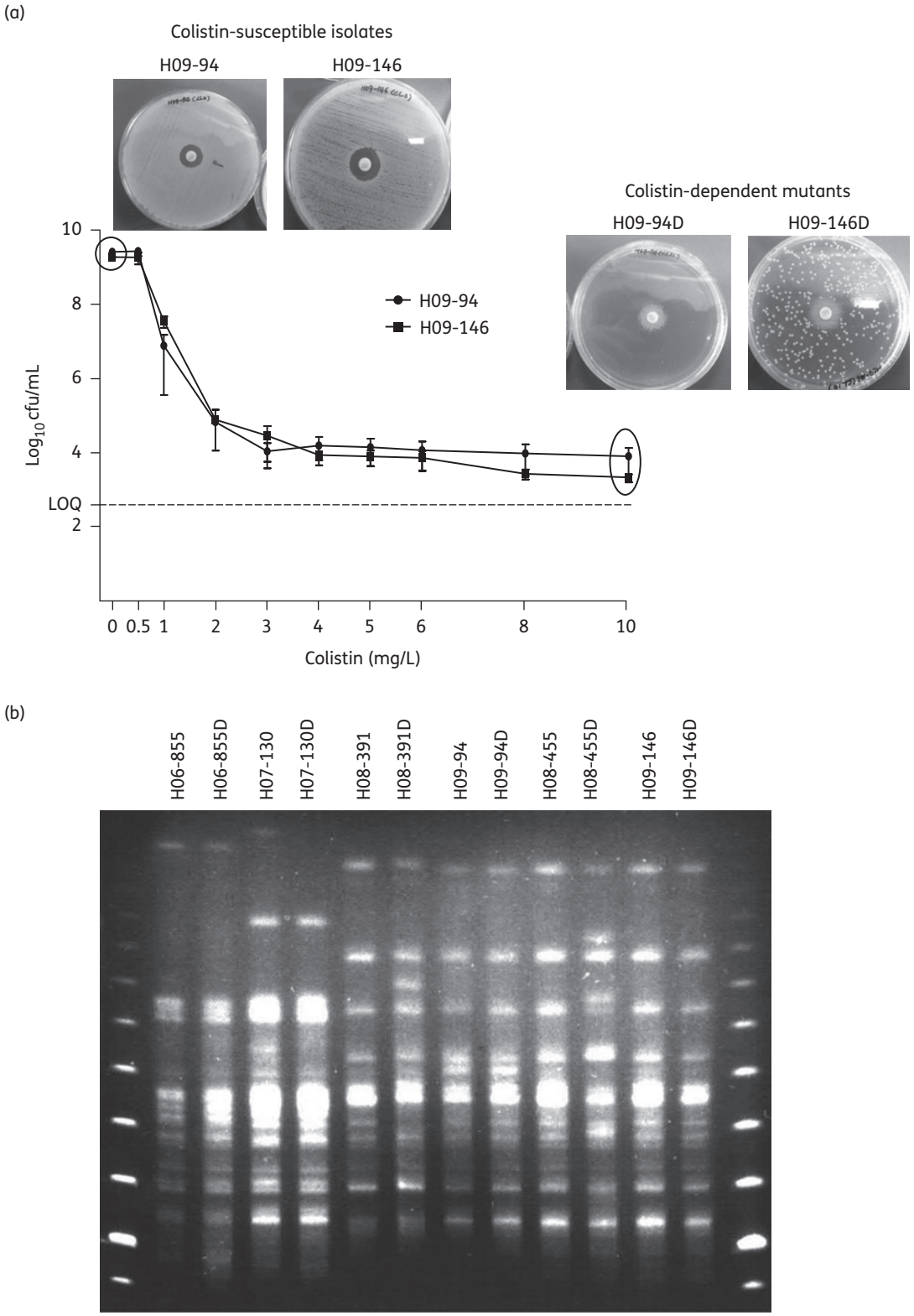

Figure 1. (a) Population analysis results and the development of colistin dependence in colistin-susceptible *A. baumannii* isolates H09-94 and H09-146. The experiments with the isolates were performed twice. Results of the disc diffusion test indicated that colonies surviving at 0 mg/L colistin had 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.[4,5] 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

None to declare.

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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.[1] Although phenotypically resistant to methicillin, *mecC* isolates typically yield negative results for conventional *mecA* PCR and the PBP2a assay[1] and can therefore hamper diagnosis of MRSA infections. First reported in *mecA*-negative MRSA isolates from humans and cattle,[1] *mecC* has since been found in MRSA isolated from livestock, wildlife and companion animals, including domestic cats.[2,3] Reports of *mecC* have hitherto been restricted to Europe.[4]

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.[5] 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 speciated by MALDI-TOF and identification of the species-specific *nuc* 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.[6,7] 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* type XI element. *De novo* contigs were blasted against an SCC*mec* type XI reference (*mecC* MRSA LGA251, GenBank accession number FR821779.1),[1] which allowed identification of the *mecC* type 8 *ccr* (*ccrA1* and *ccrB3*) genes. The *mecC* gene had 96% and 100% sequence similarity to the *mecC* described in *Staphylococcus aureus* LGA251[1] and *Staphylococcus sciuri* GVGS2,[8] 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.[1] The isolate did not harbour the human immune evasion *sak*, *chp* and *scn* genes,[9] 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