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Letter to the Editor

Plasmid-encoded resistance to trimethoprim/sulfamethoxazole mediated by *dfrA1*, *dfrA5*, *sul1* and *sul2* among *Acinetobacter baumannii* isolated from urine samples of patients with severe urinary tract infection

Sir,

Recently *Acinetobacter baumannii* has become a significant aetiological agent in nosocomial infections owing to its propensity to develop or acquire myriad mechanisms of multidrug resistance [1]. Due to the rapid spread of drug resistance in *A. baumannii*, there is currently a need to rely on the available old antibiotics of choice such as trimethoprim/sulfamethoxazole (SXT, cotrimoxazole) owing to the lack of novel antibacterial agents in recent years. SXT exerts a synergistic effect by inhibition of consecutive steps in the folate pathway [2]. In recent years, resistance to SXT is also an alarming threat and has created a need for antibiogram monitoring. The present investigation thus aimed to assess the prevalence of SXT resistance among *A. baumannii* isolated from urine specimens and to detect the frequency of plasmid-borne genetic determinants for SXT resistance. Over a 12-month period, 73 consecutive, non-duplicate *A. baumannii* isolates from urine samples of patients with severe urinary tract infection ($n = 1000$) exhibiting one or more of the following symptoms among frequency and urgency of urination, suprapubic discomfort, dysuria and flank pain were included in this study. Appropriate ethical clearance and informed consent were obtained from the study cases in accordance with standard guidelines. All of the strains were identified both by phenotypic assay using biochemical characterisation assays [3] and by genotypic characterisation using a standard protocol with forward (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse (5′-TACCAGGG-TATCTAATCCTGTT-3′) primers that produced an amplicon of size 750 bp. The susceptibility of *A. baumannii* to trimethoprim/sulfamethoxazole (1.25/23.75 μg) was determined by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2012) using antibiotic disks from HiMedia (Mumbai, India). Furthermore, all of the *A. baumannii* isolates were maintained at −80 °C in 80/20% (v/v) glycerol in Luria–Bertani medium, and plasmids were extracted using a QIAGEN Plasmid Mini Kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. Amplification of the SXT resistance determinants *dfrA1*, *dfrA5*, *sul1* and *sul2* was performed by PCR (Fig. 1). Randomly selected PCR products of *sul1*, *sul2*, *dfrA1* and *dfrA5* genes were outsourced for sequencing. The susceptibility profile of the *A. baumannii* isolates to SXT showed resistance in 91.8% (67/73), intermediate resistance in 2.7% (2/73) and susceptibility in 5.5% (4/73) (Table 1). PCR amplification of the genetic determinants for SXT resistance showed the presence of plasmid-borne *dfrA1*, *dfrA5*, *sul1* and *sul2* in 34.2% (25/73), 32.9% (24/73), 52.1% (38/73) and 45.2% (33/73), respectively. Moreover, 23.3% (17/73) of the isolates showed the presence of both *dfrA1* and *dfrA5*, 37.0% (27/73) showed the presence of both *sul1* and *sul2*, and 17.8% (13/73) showed the presence of all four genetic determinants, whereas 35.6% (26/73) of the isolates were PCR-negative. The four susceptible isolates did not yield any target genes under study. Among the 67 resistant isolates, 22 isolates showed negative PCR for the target genes under study. The *sul1* sequence of *A. baumannii* from the present study when compared with plasmids of *A. baumannii* reference strains B11911 (pB11911), HWBA8 (pHWBA8_1) and 255_n (p255n_1) in the database showed high similarity with the observed sequence, but not with the Ab8098 (pAb8098) plasmid that showed deletions and mismatches compared with the sequence from the current study. The *sul2* sequence alignment also showed similarity of *A. baumannii* with plasmid sequences from strains D46 (pD46-4), 7804 (pAba7804b), B11911 (pB11911) and HWBA8 (pHWBA8_1). Similarly, the *dfrA1* sequence was aligned with the genome of *A. baumannii* strains AYE, IR-TUMS/AbaR, B-389/14 class 2 integron and A32 class 2 integron In2-4, which confirmed identity of the *dfrA1* gene in the present study. Furthermore, the *dfrA5* gene was subjected to multiple sequence alignment with the genome of *A. baumannii* strains A85, AB0057, USA15 and A1, which showed the highest identity to the ribonucleotide diphosphate reductase subunit [4]. In the present study, a high frequency (91.8%) of resistant strains was observed among the tested isolates, with 2.7% intermediate-resistant isolates. With many reports suggesting the variation in the susceptibility pattern to SXT among Gram-negative bacteria, the current study also correlates with the context of a high frequency of the four important genetic determinants, namely *dfrA1*, *dfrA5*, *sul1* and *sul2*. The relatedness of these genes in inducing SXT resistance is common and the frequency of distribution in inducing the resistance pattern varies in the literature [5]. This variation is because of the genetic relatedness of the target genes in the varying gene cassettes in integrons and its varying pattern of association with insertion sequences that are commonly observed among uropathogenic Gram-negative bacteria [6]. In the present investigation, among the genes associated with SXT resistance, 26 (35.6%) of the isolates were PCR-negative for *dfrA1*, *dfrA5*, *sul1* and *sul2* genes even though they were resistant for SXT. This might be due to resistance conferred by other groups of *dfr* and *sul* genes that were not included in the study. Multiple sequence alignment of the proposed genes showed high sequence homology with reference sequences retrieved from the database. These data suggests that *A. baumannii* is still evolving with more potent antimicrobial resistance mechanisms. This warrants further investigations to uncover the genetic relatedness with the obtained and existing reports related with these data. However, the present investigation has helped us to update the existing pattern of SXT resistance along with the genetic relatedness of *dfrA1*, *dfrA5*, *sul1* and *sul2* genes in

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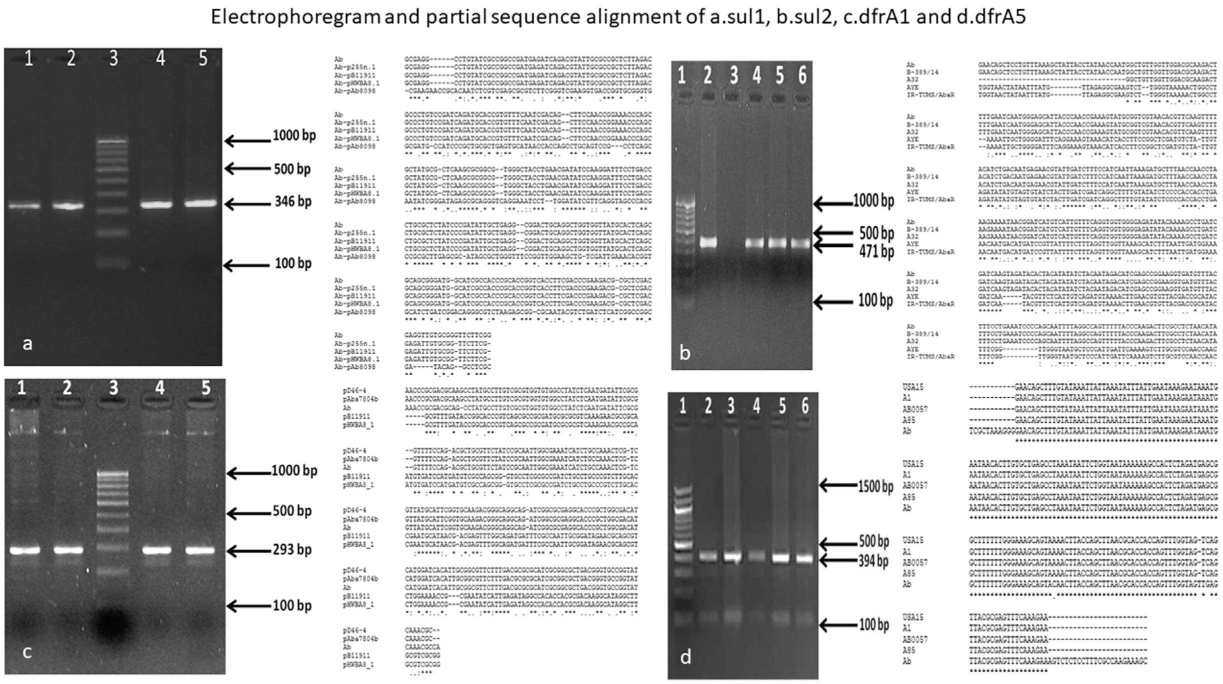

Fig. 1. Electropherogram and partial sequence alignment of (a) *sul1*, (b) *dfrA1*, (c) *sul2* and (d) *dfrA5*.

Table 1
Susceptibility profile of *Acinetobacter baumannii* strains ($n = 73$) to trimethoprim/sulfamethoxazole (1.25/23.75 μg) by the Kirby–Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2012).

	S	I	R
Interpretive criteria (zone diameter to nearest whole mm)	≥16	11–15	≤10
% of isolates (*n*)	5.5% (4)	2.7% (2)	91.8% (67)

S, susceptible; I, intermediate; R, resistant.

inducing the same. Analysing the data, it is evident to adopt prudent use of antimicrobial agents with the establishment of a periodical surveillance system for older antibiotics such as SXT in order to curb further dissemination of the resistance pattern among *A. baumannii* in the hospital environment.

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Competing interests

None declared.

Ethical approval

Appropriate ethical clearance and informed consent were obtained from the study cases in accordance with standard guidelines.

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