

LETTER



Decreased susceptibility to chlorhexidine affects a quarter of *Escherichia coli* isolates responsible for pneumonia in ICU patients

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Dear Editor,

The effectiveness of chlorhexidine (CHX) oral care in the prevention of ventilator-associated pneumonia (VAP) has been questioned in non-cardiac surgery patients [1]. *Enterobacteriaceae* (including *Escherichia coli*) are increasingly responsible for VAP [2], and according to the World Health Organization, *E. coli* is one of the critical-priority antibiotic-resistant bacteria. Surprisingly, there is no data on CHX susceptibility of *E. coli* pneumonia isolates. We aimed to study CHX and antibiotic susceptibility of intensive care unit (ICU) *E. coli* pneumonia isolates, as a correlation between antibiotic resistance and decreased CHX susceptibility has been described for other pathogens [3].

We studied 260 *E. coli* pneumonia isolates retrieved from mechanically ventilated patients in 14 French ICUs (ColoColi study [4], ClinicalTrials.gov identifier NCT03303937). This study was approved by the ethics

committee of the French Intensive Care Society (SRLF-CE 12-388). To study how effective CHX was in inhibiting bacterial growth, we exposed each isolate to 2 mg/L CHX (Sigma-Aldrich, Saint-Quentin Fallavier, France) in lysogeny broth medium (this concentration corresponds to the residual salivary concentration of CHX measured in healthy volunteers [5]). We measured bacterial growth over a 24-h period with an automatic spectrophotometer that generated growth curves. We considered the maximal growth rate (MGR; see Electronic Supplemental Material [ESM], Fig. 1) in the presence of CHX 2 mg/L, expressed in h⁻¹. The antibiotic susceptibility of each isolate was determined by the disk-diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Tested antibiotics are detailed in Fig. 1. The resistance score was defined as the sum of inactive antibiotics (intermediate + resistant) in vitro for each isolate. Analyses were performed using GraphPad Prism7 software (GraphPad Software, San Diego, CA, USA).

In the presence of CHX 2 mg/L, two *E. coli* populations were distinguishable by their susceptibility to CHX

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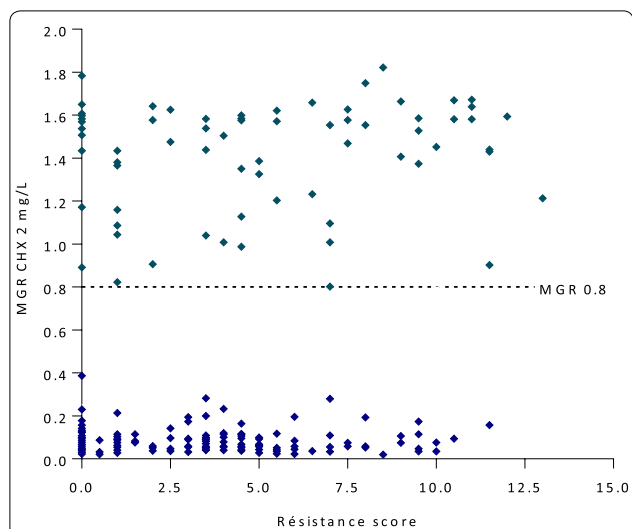


Fig. 1 Maximal growth rate (MGR) of *E. coli* isolates in the presence of chlorhexidine (CHX) 2 mg/L, according to antibiotic resistance score. Resistance score is defined as the sum of inactive in vitro antibiotics for each isolate. The antibiotics tested were tobramycin, netilmicin, kanamycin, gentamicin, amikacin, doxycycline, minocycline, nalidixic acid, ofloxacin, ciprofloxacin, fosfomicin, furans, trimethoprim, amoxicillin, amoxicillin–clavulanate, ticarcillin, piperacillin, piperacillin–tazobactam, imipenem, cefalotin, ceftazidime, cefepime. Two very distinct populations of *E. coli*, depending on their MGR, can be seen. Three quarters of the entire collection had an MGR below 0.5 h^{-1} , indicating very little if any growth when exposed to CHX. In contrast, a quarter of the collection had MGR above 0.8 h^{-1} , showing an ability to grow despite exposure to CHX

(Fig. 1). Over a quarter of the strains ($n = 70$, 26.9%) had a high MGR (above 0.8 h^{-1}), suggesting that CHX was less effective in inhibiting the growth of these isolates than the remaining 190 isolates with an MGR below 0.4 h^{-1} . The median resistance score was 4 (0–8; see ESM). There was a weak but significant correlation between the CHX MGR of the isolates and their resistance scores ($r = 0.17$, $p = 0.007$), with MGR ranging from 0.02 to 1.82 h^{-1} . More precisely, the resistance of strains to quinolones, amoxicillin, cephalosporins, gentamicin, tobramycin, or tetracyclines was significantly correlated with CHX MGR (ESM E2). Similarly, the CHX MGR of isolates producing an extended-spectrum beta-lactamase was significantly higher than that of the wild-type isolates (0.17 vs. 0.08 , $p = 0.01$).

We report for the first time the CHX susceptibility profile of a large collection of pneumonia *E. coli* isolates retrieved from mechanically ventilated patients. A quarter of the isolates grew despite exposure to CHX 2 mg/L, the most widely used oral care antiseptic for the prevention of VAP. Possible resistance mechanisms are being investigated. In agreement with a recent meta-analysis and expert comments [1], our results may call into question the use of CHX in VAP prevention bundles, and may

also provide some clue for the negative results of studies evaluating CHX in this indication.

Electronic supplementary material

The online version of this article (<https://doi.org/10.1007/s00134-018-5061-8>) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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