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# Epidemiological and Clinical Complexity of Amoxicillin-Clavulanate-Resistant *Escherichia coli*

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**Two hundred twelve patients with colonization/infection due to amoxicillin-clavulanate (AMC)-resistant *Escherichia coli* were studied. OXA-1- and inhibitor-resistant TEM (IRT)-producing strains were associated with urinary tract infections, while OXA-1 producers and chromosomal AmpC hyperproducers were associated with bacteremic infections. AMC resistance in *E. coli* is a complex phenomenon with heterogeneous clinical implications.**

Amoxicillin-clavulanate (AMC) is one of the most frequently used antimicrobials in many countries (1, 2). Over the last years, an increase in the rate of resistance to AMC has been noted among *Escherichia coli* isolates in several areas (3–5), which has been related to an increase in the consumption of this antibiotic (3). However, to our knowledge, there are no recent clinical studies investigating infections caused by AMC-resistant *E. coli*. The objective of this study is to analyze the clinical epidemiology and features of infections caused by AMC-resistant *E. coli*.

A cross-sectional multicenter study was performed in 7 Spanish tertiary centers. Each center was committed to include up to 44 patients from whom AMC-resistant *E. coli* (MIC of ≥32 [amoxicillin]-16 [clavulanate] mg/liter and/or disk inhibition zone of ≤13 mm) were isolated from clinical samples (22 community-onset and 22 nosocomial isolates) from 15 January to 15 May 2010. Overall, 257 AMC-resistant *E. coli* isolates were collected. A previous publication reported the mechanism of resistance to AMC and clonality for all these isolates (6). For the present analysis, 13 patients harboring isolates with more than one mechanism of resistance to AMC, 3 patients harboring isolates for which the mechanism of resistance was not elucidated, and 37 patients with isolates producing extended-spectrum β-lactamases (ESBLs) were excluded; thus, 212 patients are studied here. Epidemiological and clinical data were collected by reviewing the clinical charts and interviewing the patients or their relatives. The study was approved by the ethics committees of the participating centers. Isolates were considered nosocomial if obtained from patients who were hospitalized for >48 h. Community isolates were classified as health care associated according to previously described criteria (7). CDC criteria (8) were used for establishing whether the patients had an infection and the types of infection.

The microbiological methods used were previously reported in detail (6). Briefly, antibiotic susceptibility was confirmed by microdilution according to CLSI recommendations (9). The mechanisms of resistance to AMC of the 212 isolates included here, as determined from the previously reported data (6), were as follows: hyperproduction of TEM-1 or SHV-1 (HPen) (54 isolates [25.5%]), production of inhibitor-resistant TEM (IRT) (43 [20.3%]), hyperproduction of chromosomal AmpC (c-AmpC) (40 [18.9%]), production of plasmidic AmpC (p-AmpC) (40 [18.9%]), and production of OXA-1 (35 [16.5%]). Isolates showing resistance to at least one of the following families/drugs (in addition to AMC and ampicillin) were considered multidrug resistant: aminoglycosides, cephalosporins, carbapenems, piperacillin-tazobactam, ciprofloxacin, and trimethoprim-sulfamethoxazole (10). The number of above-mentioned families/drugs to which each isolate was not susceptible (resistance score) was calculated. Proportions with 95% confidence intervals (CIs) were used to compare categorical variables. Multivariate analyses were performed by logistic regression analysis; variables were selected by a stepwise backward method.

The microbiological features of the 212 isolates included are shown in Table S1 in the supplemental material. c-AmpC hyperproducers and OXA-1 producers belonged more frequently to phylogroup A1, while HPen, IRT, and p-AmpC producers more frequently belonged to phylogroup B2. HPen and OXA-1 producers were less susceptible to piperacillin-tazobactam; OXA-1-producers were also less susceptible to ciprofloxacin, gentamicin, tobramycin, and trimethoprim-sulfamethoxazole; and c-AmpC hyperproducers and p-AmpC producers were less susceptible to

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**TABLE 1** Exposure to predisposing factors among amoxicillin-clavulanate-resistant *E. coli* isolates according to mechanism of resistance[a]

| Predisposing factor | % of patients (95% CI) harboring isolate with mechanism of resistance | | | | | |
|---|---|---|---|---|---|---|
| | All cases ($n = 212$) | HPen ($n = 54$) | c-AmpC ($n = 40$) | p-AmpC ($n = 40$) | IRT ($n = 43$) | OXA-1 ($n = 35$) |
| Male gender | 33 (27–40) | 35 (24–48) | 22 (12–37) | 35 (22–50) | 30 (19–45) | 43 (28–59) |
| Age >65 yr | 52 (45–58) | 41 (29–54) | 22 (12–37) | 22 (12–37) | 42 (28–57) | 68 (52–81) |
| Type of acquisition | | | | | | |
| Nosocomial | 42 (35–49) | 63 (50–74) | 30 (18–45) | 45 (31–60) | 32 (20–47) | 31 (18–48) |
| Health care related | 24 (18–30) | 9 (4–20) | 30 (18–45) | 32 (20–48) | 30 (19–45) | 20 (10–36) |
| Community | 34 (28–41) | 28 (18–41) | 40 (26–55) | 22 (12–37) | 37 (24–52) | 48 (33–64) |
| Nursing home resident | 7 (4–11) | 2 (0–10) | 10 (4–23) | 12 (5–26) | 7 (2–19) | 3 (0–14) |
| Diabetes mellitus | 19 (14–25) | 22 (13–25) | 27 (16–43) | 12 (5–26) | 16 (8–20) | 14 (6–29) |
| Chronic pulmonary disease | 10 (7–15) | 17 (9–29) | 12 (5–26) | 2 (0–13) | 9 (4–22) | 8 (3–22) |
| Chronic renal insufficiency | 12 (8–17) | 13 (6–24) | 10 (4–23) | 12 (5–26) | 12 (5–24) | 14 (6–29) |
| Chronic liver disease | 6 (3–10) | 7 (3–17) | 5 (1–16) | 5 (1–16) | 9 (4–22) | 0 (0–10) |
| Malignancy | 19 (14–25) | 22 (13–25) | 10 (4–23) | 20 (10–35) | 14 (6–27) | 28 (16–45) |
| Urinary catheter | 20 (15–26) | 30 (19–43) | 17 (9–32) | 27 (16–43) | 9 (4–22) | 14 (6–29) |
| Previous antibiotic use | 58 (52–65) | 61 (48–73) | 60 (45–74) | 67 (52–80) | 58 (43–72) | 43 (28–59) |
| Amoxicillin-clavulanate | 26 (21–33) | 28 (18–41) | 30 (18–45) | 22 (12–37) | 35 (22–50) | 14 (6–29) |
| Piperacillin-tazobactam | 9 (6–14) | 28 (18–41) | 2 (0–13) | 0 (0–9) | 16 (8–20) | 3 (0–14) |
| Carbapenems | 8 (5–12) | 11 (5–22) | 5 (1–16) | 10 (4–23) | 16 (8–20) | 6 (1–19) |
| Cephalosporins | 7 (5–12) | 7 (3–17) | 0 (0–9) | 10 (4–23) | 12 (5–24) | 8 (3–22) |
| Fluoroquinolones | 19 (15–25) | 17 (9–29) | 20 (10–35) | 22 (12–37) | 19 (10–33) | 20 (10–36) |
| Aminoglycosides | 4 (2–7) | 6 (2–15) | 2 (0–13) | 2 (0–13) | 7 (2–19) | 0 (0–10) |

[a] HPen, TEM-1 or SHV-1 hyperproducer; IRT, inhibitor-resistant TEM producer; c-AmpC, chromosomal AmpC hyperproducer; p-AmpC, plasmidic AmpC producer; OXA-1, OXA-1 producer.

cefotaxime and ceftazidime. IRT producers were less frequently multidrug resistant.

Data for exposure to predisposing factors are shown in Table 1. Nosocomial acquisition and previous exposure to piperacillin-tazobactam were more frequent among HPen isolates. When the susceptibility of the specific isolate to the previously used antimicrobial in each patient was considered, a significant association was found for piperacillin-tazobactam (a resistant strain was isolated in 14/20 [70%] patients exposed to this antimicrobial, versus 39/192 [35.9%] patients not exposed; relative risk [RR] = 1.96; 95% CI, 1.38 to 2.74; $P = 0.003$) and ciprofloxacin (30/41 [73.2%] versus 82/171 [48%]; RR = 1.52; 95% CI, 1.19 to 1.94; $P = 0.004$) but not for other antimicrobials (data not shown).

The types of infections are shown in Table 2. Urinary tract infections (UTIs) and specifically cystitis were somehow more frequent among IRT and OXA-1 producers, particularly in comparison to HPen. On the contrary, skin and soft tissue infections were more frequent among HPen isolates. We further investigated whether some specific mechanism of resistance was independently associated with urinary tract infection as opposed to other infections and to the occurrence of bacteremia by using logistic regression analysis. The final models are shown in Table S2 in the supplemental material. In summary, IRT and OXA-1 producers of the B2 phylogroup were associated with UTI, and c-AmpC hyperproducers and OXA-1 producers were associated with bacteremic infections. Among bacteremic infections, all 4 caused by OXA-1 producers and 2 out of the 6 caused by c-AmpC hyperproducers belonged to phylogroup A.

These data, together with those focused on the molecular aspects previously reported (6), show that AMC resistance in *E. coli* is a complex and heterogeneous phenomenon from both molecular and clinical points of view. We hypothesize that the recent increase in AMC consumption triggered the spread of diverse mechanisms of resistance that were initially limited to some isolates or clones in specific epidemiological niches.

The epidemiologic features of the patients infected with AMC-resistant isolates were somehow similar to those found among patients harboring ESBL-producing or p-AmpC-producing *E. coli* (7, 11); a more specific finding is the frequent previous exposure to β-lactam–β-lactam inhibitors, particularly AMC. Of note, patients with HPen isolates were significantly more frequently exposed to piperacillin-tazobactam, in relation with the fact that HPen isolates were also more frequently resistant to this antimicrobial (patients with OXA-1-producing isolates, which were also frequently resistant, were less exposed to this antimicrobial because of a predominance of nonnosocomial infections). Previous use of fluoroquinolones was associated with isolation of a strain resistant to these drugs; this is another example of the strong co-selection activity of fluoroquinolones (12).

Regarding the differences across the specific AMC-related mechanisms of resistance, OXA-1-producing isolates were mostly from phylogroup A and showed the highest resistance score but,

**TABLE 2** Types of infection caused by amoxicillin-clavulanate acid-resistant *E. coli* isolates according to mechanism of resistance[a]

| Infection | % of patients (95% CI) harboring isolate with mechanism of resistance | | | | | |
|---|---|---|---|---|---|---|
| | All cases ($n$ = 212) | HPen ($n$ = 54) | IRT ($n$ = 43) | c-AmpC ($n$ = 40) | p-AmpC ($n$ = 40) | OXA-1 ($n$ = 35) |
| Urinary tract | 67 (60–73) | 46 (34–59) | 79 (65–88) | 65 (49–78) | 67 (52–80) | 86 (71–94) |
| Asymptomatic bacteriuria | 5 (3–10) | 0 (0–7) | 5 (1–15) | 7 (2–20) | 15 (7–29) | 3 (0–14) |
| Cystitis | 59 (53–66) | 44 (32–58) | 74 (60–85) | 55 (40–69) | 50 (35–65) | 80 (64–90) |
| Pyelonephritis/prostatitis | 2 (1–5) | 2 (0–10) | 0 (0–8) | 2 (0–13) | 2 (0–13) | 3 (0–14) |
| Intra-abdominal infection | 7 (4–12) | 17 (9–29) | 5 (1–15) | 5 (1–16) | 2 (0–13) | 6 (1–19) |
| Skin and soft tissue infection | 14 (10–19) | 28 (18–41) | 9 (4–21) | 7 (2–20) | 12 (5–26) | 6 (1–19) |
| Respiratory tract infection | 3 (1–6) | 2 (0–10) | 5 (1–15) | 5 (1–16) | 2 (0–13) | 0 (0–10) |
| Primary bacteremia | 4 (2–7) | 2 (0–10) | 0 (0–8) | 10 (4–23) | 5 (1–16) | 3 (0–14) |
| Others | 5 (3–9) | 6 (2–15) | 2 (0–12) | 7 (2–20) | 10 (4–23) | 0 (0–10) |
| Bacteremia (primary or secondary) | 7 (5–12) | 6 (2–15) | 2 (0–12) | 15 (7–29) | 5 (1–16) | 11 (4–30) |

[a] HPen, TEM-1 or SHV-1 hyperproducer; IRT, inhibitor-resistant TEM producer; c-AmpC, chromosomal AmpC hyperproducer; p-AmpC, plasmidic AmpC producer; OXA-1, OXA-1 producer.

unexpectedly, were associated with both UTI and bacteremic infections. We recently showed that phylogroup A isolates were also more frequent than expected among bacteremic ESBL-producing *E. coli* isolates in Spain (13). We hypothesize that previous antibiotic use would select for these less virulent (14), multidrug-resistant strains, which hence may cause invasive infections in predisposed patients. IRT-producing strains were also associated with UTI, but this was expected because they belonged predominantly to phylogroup B2; finally, c-AmpC hyperproducers were also associated with bacteremic infections.

Our study has some limitations. We did not include a control group formed by AMC-susceptible isolates. Outcome data were not collected. Finally, the epidemiology of AMC-resistant *E. coli* might be different in other areas. Some strengths of our work include the multicenter nature and the combined analysis of molecular and clinical data.

In conclusion, AMC resistance in *E. coli* is a heterogeneous and complex phenomenon of clinical importance. More studies are needed to elucidate the importance of drivers potentially implicated in the recent increase in the rate of resistance to AMC.

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