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Short Communication

Epidemiology of Extended-Spectrum β-Lactamase, AmpC, and Carbapenemase Production in *Proteus mirabilis*

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SUMMARY: *Proteus mirabilis* strains that produce extended-spectrum β-lactamase (ESBL), AmpC β-lactamase, and carbapenemase pose potential threats to patient care because most clinical diagnostic laboratories may not attempt to detect these three major groups of enzymes. Therefore, the objective of this study was to ascertain if *P. mirabilis* isolates collected from our heathcare facility possess various mechanisms of resistance to β-lactams (i.e., ESBL, AmpC, and carbapenemases) and to additionally arrive at conclusions regarding concurrent testing for these three mechanism of drug resistance in order to reduce cost and time in routine diagnostic testing. Between January 2011 and June 2011, 60 consecutive non-repeated strains of *P. mirabilis* were evaluated for production of ESBLs, AmpC β-lactamases, and carbapenemases. Of these, 36 isolates were found to be ESBL producers, and 7 (12%) were positive for production of AmpC β-lactamases and ESBLs. Therefore, 19.4% of ESBL-producing *Proteus* strains coproduced AmpC enzymes. The modified Hodge test confirmed carbapenemase production in only 1 isolate (1.7%), which was also ESBL- and AmpC-positive. The clinical impact of additional AmpC expression in ESBL-producing *P. mirabilis* results in a newly acquired resistance to β-lactamase inhibitors. In addition, to save time and costs, we recommend the use of cefepime/cefepime-clavulanate or boronic acid for the ESBL detection but in only those strains that were positive for ESBL by screening and negative by confirmatory tests.

Proteus mirabilis is an emerging cause of nosocomial infections, particularly of wounds and the urinary tract. The various types of *P. mirabilis* infections are difficult to treat because of simultaneous production of enzymes, such as extended-spectrum β-lactamases (ESBLs), AmpC, and carbapenemases (1–3).

The Clinical Laboratory Standards Institute (CLSI) guidelines for the detection of ESBLs in *Enterobacteriaceae* are suitable to address the routine work flow of most microbiology laboratories (4). The incidence of *P. mirabilis* known to harbor both ESBL and plasmid-mediated AmpC β-lactamases is estimated to increase healthcare costs worldwide. AmpC are enzymes, which preferentially hydrolyze cephalosporins and cephamycins and are not inhibited by clavulanate, sulbactam, or tazobactam (5). In addition, the detection of AmpC in ESBL-positive isolates is challenging because high AmpC levels produce false-negative results in ESBL confirmatory tests, which can be avoided by methods unaffected by AmpC β-lactamases (5).

The treatment of choice for ESBL-positive isolates includes carbapenem and β-lactam/β-lactamase inhibitors. In addition, for uncomplicated infections, such as urinary tract infections, the choice of antibiotics includes ciprofloxacin, nitrofurantoin, and fosfomycin. Due to the inoculum effect during the treatment with β-lactam/β-lactamase inhibitors, carbapenems are the last resort for treatment of ESBL-associated infections. In addition, organisms producing AmpC are treated best with cefepime or carbapenem (5). Carbapenem resistance in *Enterobacteriaceae* may be due to overproduction of AmpC or ESBL in organisms with porin mutations or due to production of carbapenemases (6). The pathogens harboring these enzymes may not be recognized during routine diagnostic laboratory testing because they give false-susceptibility results in routine testing (5). In addition, most clinical diagnostic laboratories may not attempt to detect all the three major enzyme groups (i.e., ESBLs, AmpC, and carbapenemases) simultaneously in *Enterobacteriaceae* spp. (5). Therefore, the objective of this study was to ascertain if *P. mirabilis* isolates collected from our heathcare facility possess the various mechanisms of resistance to β-lactams (such as ESBLs, AmpC, and carbapenemases) and to additionally arrive at conclusions regarding concurrent testing for these three mechanisms of drug resistance in order to save costs and time associated with routine diagnostic testing.

Between January 2011 and June 2011, all consecutive non-repeated strains of *P. mirabilis* isolated from various clinical samples in the Department of Microbiology were evaluated. The samples included wound swabs, drained fluids, tracheal aspirate and peritoneal fluids, pleural fluids, and vaginal swabs. *P. mirabilis* was identified by characteristic growth on blood and MacConkey agar plates and various biochemical tests (7).

Routine antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion test according to CLSI criteria for all *P. mirabilis* strains on Mueller–Hinton agar (Hi-Media, Mumbai, India). The antibiotics tested include ciprofloxacin (5 μg), amikacin (30 μg),

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cefoperazone/sulbactam and piperacillin/tazobactam (100/10 μg), cefepime (30 μg), cefotaxime (3 μg), ceftazidime (30 μg), cefoxitin (30 μg), meropenem (10 μg), and ertapenem (10 μg) (Hi-Media).

Furthermore, all *P. mirabilis* isolates were tested for production of ESBLs, AmpC β-lactamases, and carbapenemases. Only those strains that were positive by screening tests were further subjected to confirmatory tests.

An inhibition zone diameter of *P. mirabilis* of <22 mm for ceftazidime and of ≤ 27 mm for cefotaxime presumptively indicates ESBL production (4). Confirmatory testing for ESBL was performed according to CLSI criteria (4).

Presumptive tests for inducible AmpC β-lactamases was considered positive if the inhibition zone diameter of *P. mirabilis* for cefoxitin was ≤ 18 mm (5). AmpC β-lactamase production was confirmed by the AmpC disk test as described by Black et al. (8). All strains of *P. mirabilis*-positive for AmpC by the above two methods was confirmed using Etest strips (cefoxitin/cefoxitin + cloxacillin; AB Biodisk, Solna, Sweden). Either a reduction in the MIC of cephamycin by at least three dilutions, deformation of the ellipse of inhibition, or a phantom zone was interpreted as a positive test result (9).

If any of the strains had negative or inconclusive results for the CLSI ESBL confirmatory test but showed reduced susceptibility to third generation cephalosporin and was positive for AmpC, we tested for ESBL production by the cefepime/cefepime-clavulanate Etest, which is not affected by AmpC (5).

Presumptive tests for carbapenemase susceptibility were considered positive if the inhibition zone diameter of *P. mirabilis* was ≤ 19 mm for meropenem and ≤ 19 mm for ertapenem (4). The modified Hodge test (MHT) was performed to confirm the presence of carbapenemases (4).

Over a 6-month period, a total of 60 strains of *P. mirabilis* were isolated from various samples and assessed for the production of ESBLs, AmpC, and carbapenemases. Of the 60 strains of *P. mirabilis*, 36 (60%) were found to be ESBL producers by both screening and confirmatory tests. Eight of the strains were cefoxitin-resistant; however, confirmation by the AmpC disk test showed that 7 (12%) were positive for AmpC β-lactamase production (Table 1). All the 7 AmpC-positive strains were also ESBL-positive. Therefore, 19.4% of ESBL-producing *Proteus* strains also coproduced the AmpC enzyme. However, none of our strains showed reduced susceptibility to expended-spectrum cephalosporin (positive-ESBL screen), whereas the ESBL confirmatory test results were either negative or inconclusive. Therefore, the cefepime/cefepime–clavulanate Etest was not necessary for the detection of ESBL in our AmpC-positive strains. Carbapenemase production was detected in only 1 isolate (1.7%) by MHT, which initially showed decreased sensitivity to both ertapenem and meropenem (Table 1). The *P. mirabilis* strain that produced carbapenemases also coproduced ESBL and AmpC.

ESBL-producing strains were more resistant to non-β-lactam drugs than non-ESBL-producing strains. Ciprofloxacin sensitivity among the ESBL producers was 69.7% compared with 86.7% among the non-ESBL producers. In addition, 37.5% of ESBL producers were sensitive to amikacin, 13% to cefepime, 40.9% to cefoperazone/sulbactam, and 45.5% to piperacillin/tazobactam. In contrast, among the non-ESBL producers, sensitivities were 92.3%, 84.6%, 100%, and 100%, respectively. Among the AmpC-producing isolates, 62.5% were sensitive to ciprofloxacin, 14.3% to amikacin, 16.7% to cefepime, and 20% to cefoperazone/sulbactam and piperacillin/tazobactam. Carbapenem was the most effective agent against these isolates, followed by ciprofloxacin, cefoperazone/sulbactam and piperacillin/tazobactam, cefepime, and amikacin.

Table 1. Number of isolates of *Proteus mirabilis* producing ESBL, AmpC, and carbapenemase

Test	No. (%) of isolate positive ($n = 60$)
ESBL screening test	36 (60)
ESBL confirmatory test	36 (60)
AmpC screening test	8 (13)
AmpC confirmatory test	7 (12)
Carbapenemase screening test	1 (1.7)
Carbapenemase confirmatory test	1 (1.7)

Rudresh et al. reported 57.1% of ESBL-producing and 28% of AmpC-producing *Proteus* strains (10), whereas Khan et al. demonstrated that 44% of *P. mirabilis* strains were ESBL producers and 37% were AmpC producers (11). Among the *Proteus* isolates collected from our hospitals, the rate of AmpC production was low compared with other reports. The higher degree of antibiotic coresistance amongst ESBL producers suggested the importance of detecting ESBL production and prudent use of antibiotics.

We observed that one cefoxitin-resistant isolate did not produce AmpC. This may be attributed to other resistance mechanisms, such as porin channel alterations. Although some of the AmpC-producing isolates showed in vitro susceptibility to cefoperazone/sulbactam and piperacillin/tazobactam, the clinical usefulness of these agents is doubtful, as AmpC is not inhibited by β-lactamase inhibitors (9).

We found that 19.4% of our ESBL-positive strains also coproduced AmpC. Although no strain was found to produce only AmpC, the isolates producing AmpC, but not ESBL, can be effectively treated with cefepime and cefpirome. However, this choice does not appear to be possible for *Proteus* strains collected from our healthcare facility. In contrast, Rudresh and Nagarathnamma reported that 15.1% of bacterial isolates were pure AmpC producers (10).

Although high AmpC expression levels may prevent identification of ESBL-positive isolates, this was not the case in our study (12,13). ESBL in AmpC-positive isolates was detected by methods unaffected by AmpC β-lactamases, such as cloxacillin incorporation into the culture media or use of boronic acid or cefepime/cefepime-clavulanate. Thompson reviewed ESBL detection in AmpC-producing isolates and suggested the use of the above methods only if the isolates are ESBL-positive by screening tests, AmpC-positive, and ESBL-nega-

tive by confirmatory tests (5). Most studies regarding detection of AmpC in ESBL-producing *P. mirabilis* used cefepime/cefepime-clavulanate or boronic acid (11). Increasing resistance to carbapenem in *P. mirabilis* has been reported worldwide, including India. Gupta et al. reported that 8.3% of ESBL-positive *Proteus* strains were meropenem-resistant and 6.9% were imipenem-resistant (14). Prakash isolated *Proteus* strains resistant to imipenem and meropenem (15). Fortunately only 1.7% of our *Proteus* strains were carbapenem-resistant.

There were several limitations to our study. First, we were unable to perform molecular tests to confirm drug resistance phenotypes. False-positive Hodge test results may have been due to the high level of AmpC production, which could be confirmed by molecular techniques. In addition, ACC (a type of AmpC enzyme) do not confer resistance to cefoxitin or give positive AmpC disk test results; therefore, molecular testing is the only suitable detection method. Thus, we may have missed this type of AmpC enzyme. Molecular typing of *P. mirabilis* strains harboring the carbapenemase enzyme can be used to categorize the isolates (group A, B, or D) (16). Finally, clonal relatedness among ESBL-, AmpC-, and carbapenemase-positive isolates could aid in better management of these isolates.

In conclusion, microbiology laboratories must continue to confirm the production of multiple β-lactamases in bacterial isolates. Because the prevalence of ESBL and AmpC β-lactamases varies geographically, we recommend the use of cefepime/cefepime-clavulanate or boronic acid for the ESBL detection; however, testing should be limited to only those strains that are positive for ESBL by screening and negative by confirmatory tests. This will save time as well as costs in most diagnostic laboratories with limited resources.

Conflict of interest None to declare.

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