CMPT Clinical Bacteriology Program

Innovation, Education, Quality Assessment, Continual Improvement

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Challenge M134-4

February 2014

Abdominal Abscess: *Klebsiella pneumoniae* (Ertapenem-Resistant)

HISTORY

The challenge was sent to category A laboratories. The sample was a simulated intra-abdominal abscess fluid obtained from a 66 year old male patient.

Laboratories were expected to isolate, identify, and report *Klebsiella pneumoniae*. Participants were asked to report susceptibility results to third generation cephalosporins, carbapenems, aminoglycosides, trimethoprim-sulfamethoxazole (SXT), piperacillin-tazobactam, and ciprofloxacin.

CMPT QC

CMPT quality control yielded a heavy growth of *Klebsiella pneumoniae* viable for 20 days.

Although not initially intended, two different *K. pneumoniae* strains were isolated presenting different susceptibility patterns (Table 1).

SURVEY RESULTS

Reference Laboratories

Identification: 15/15 (100%) laboratories reported *Klebsiella pneumoniae* (12 indicated the isolate was an ESBL producing organism, 1 laboratory indicated the isolate was multi-resistant and 2 laboratories did not comment). Consensus was reached.

Table 1. Susceptibility patterns

	Susceptibility results*	
Antibiotic	**Strain 1**	**Strain 2**
Gentamicin	Resistant	Susceptible
Amikacin	Resistant	Susceptible
Tobramycin	Resistant	Intermediate
Piperacillin/ Tazobactam	Resistant	Intermediate
Ertapenem	Resistant	Intermediate

*Both strains were also resistant to: ampicillin, amoxicillin-clavulanic acid, cefazolin, cephalothin, cefuroxime, ceftriaxone, trimethoprim-sulfamethoxazole, and ciprofloxacin

MAIN EDUCATIONAL POINTS from M134-4

1. If evidence of more than one morphotype exists, both should be identified so that susceptibilities on each can be performed. In such cases it may occur that one is quite susceptible and the other multi-resistant. Picking the susceptible one only could have significant consequences for treatment, outcome and spread of the organism in hospital.
2. An isolate of *Enterobacteriaceae* may carry multiple beta-lactam resistance mechanisms, including ESBLs, AmpCs, and carbapenemases. It is sometimes difficult in the laboratory to determine if an isolate has an ESBL alone or is AmpC positive also, but ertapenem is a useful marker to determine altered effects on carbapenems.
3. In most cases the MIC of ertapenem is suggestive of the probable mechanism of carbapenem resistance: low or intermediate level resistance to ertapenem is usually indicative of an altered porin or efflux mechanism where the strain is susceptible using gradient endpoint or broth dilution methods to other carbapenems (imipenem, meropenem, or doripenem). When the MIC to ertapenem is high (> 16 mg/L) there is a greater likelihood that the isolate has a carbapenemase and that imipenem and meropenem will also be resistant.

Infection control notification: 12/15 (80%) laboratories indicated they would notify infection control, 2 laboratories did not report, 1 laboratory indicated it would refer. Consensus was reached.

It is presumed that the laboratory that indicated referral meant that the result would be referred to Infection control for follow up. It would be more clear to indicate that infection control would be notified.

Susceptibility testing

Piperacillin/tazobactam: 13/15 (87%) reported the strain resistant, 1 reported it susceptible, 1 did not report.

Third generation cephalosporins: all laboratories reported the isolate resistant to at least one 3rd generation cephalosporin. Regardless of the agent(s) tested, the strain gave a resistant result.

Gentamicin: 11/15 (73%) reported the isolate as resistant and 4 reported it susceptible.

Grading

Maximum grade: 24

Reporting *K. pneumoniae* was graded 4.

Reporting another organism was graded 0.

Reporting to IC was graded 4.

Not reporting to IC was graded 0.

Reporting the isolate resistant to piperacillin/tazobactam, a third generation cephalosporin, ciprofloxacin, and SXT was graded 4 for each correctly reported agent.

Reporting the isolate intermediate was downgraded to 3.

Not reporting or referring the required susceptibilities was graded 0.

Tobramycin: 12/15 (80%) reported the isolate resistant to tobramycin, 2 reported it as intermediate, and one did not report.

SXT: 15/15 (100%) reported the strain resistant.

Ciprofloxacin: 15/15 (100%) reported the strain resistant

Imipenem: 7/15 (47%) reported the strain susceptible, 3 indicated they would refer, 5 did not report.

Meropenem: 11/15 (73%) reported the strain susceptible, 2 reported it intermediate, 2 did not report.

Ertapenem: 11/15 (73%) reported resistant, 1 reported intermediate, 3 did not report.

Consensus was reached for the following antibiotic agents: piperacillin/tazobactam, third generation cephalosporins, SXT, and ciprofloxacin.

Gentamcin: 11 laboratories reported gentamicin resistant and 4 labs reported susceptible.

Tobramycin: 12 laboratories reported resistant, 2 reported intermediate, 1 did not report.

Because the two different isolated strains differed in their susceptibility to aminoglycosides, these two agents were not graded.

Participants

Identification (Table 2): 63/64 (98%) laboratories reported *Klebsiella pneumoniae* and were graded 4; 57 of those laboratories indicated the strain was resistant to multiple antibiotics. One participant reported the isolate as *Enterobacter aerogenes* and was graded 0. The laboratory that did not submit a report was graded 0.

Notification to IC (Table 3): 50/64 (78%) reporting laboratories indicated they would notify IC.

Antibiotic Susceptibility Testing (Tables 4A to 4G): the results obtained by the laboratories to the antibiotic agents graded are presented in tables 4A to 4G, please refer to these tables for grading.

Table 3. IC notification results

IC/PH notification	Total	Grade
yes	50	4
n/a, lab is Public Health	1	ungraded
no report	4	0
refer notification	9	ungraded
no report on challenge	1	0
snnp ± refer	9	ungraded
Total	**74**	

COMMENTS ON RESULTS

The large majority of laboratories performed very well on this challenge. The isolate was common so that identification should not be an issue. The one laboratory that identified *Enterobacter aerogenes* should review their methodology. Although *Kelbsiella* and *Enterobacter* are members of the same group, they are readily separated.

The primary issue for the challenge was for laboratories to identify that this isolate might be a carbapenemase producing strain. Ertapenem is more sensitive to change than either imipenem or meropenem, so that apparent ertapenem resistant isolates that also test re-

Table 2. Identification results

Reported results **	Total	Grade
Klebsiella pneumoniae, ESBL / probable ESBL / refer for ESBL testing	45	4
Klebsiella pneumoniae, possible (± AmpC) KPC/carbapenemase/ MBL producer	10	4
Klebsiella pneumoniae,± refer	6	4
Klebsiella pneumoniae, multi-drug resistant organism isolated	1	4
Klebsiella pneumoniae, refer for carbapenemase testing, as per CLSI M100 S24, routine ESBL testing is not necessary	1	4
Enterobacter aerogenes, possible carbapenemase producer, refer	1	0
no report	1	0
Sample not normally processed ± refer	9	ungraded
Total	**74**	

**5 participants reported the presence of two different strains.

Table 4. Susceptibility results

4A- Piperacillin-Tazobactam (PipT)	Total	Grade
resistant	52	4
intermediate	2	3
susceptible	2	1
snnp, ± refer (susceptibility)	6	ungraded
no report susceptibility	2	ungraded
no report on challenge	1	0
snnp, ± refer (challenge)	9	ungraded
Total	**74**	

4B- 3rd generation cephalosporins	Total	Grade
Cro resistant	25	4
Cro, Caz resistant	16	4
Cro, Caz, Ctx resistant	6	4
Ctx resistant	5	4
Cro, Ctx resistant	5	4
Caz resistant	3	4
Ctx, Caz resistant	1	4
no report susceptibility	1	0
snnp, ± refer (susceptibility)	2	ungraded
no report on challenge	1	0
snnp, ± refer (challenge)	9	ungraded
Total	**74**	

Cro: ceftriaxone; Caz: ceftazidime; Ctx: cefotaxime

4E- Carbapenems	Total
Imp (1) or Mer (2) resistant	3
Imp (7) or Mer (4) susceptible	11
Ert resistant	7
Ert intermediate	1
Imi, Mer susceptible	1
Imi (1)or Mer (7) susceptible + Ert resistant	8
Mer susceptible, Ert intermediate	2
Mer, Ert resistant	8
Mer intermediate, Ert resistant	1
Imi, Mer susceptible, Ert resistant	11
Imi susceptible, Mer intermediate, Ert resistant	2
Imi susceptible, Mer, Ert resistant	1
Imi, Mer, Ert resistant	4
snnp, ± refer (susceptibility)	4
no report on challenge	1
snnp, ± refer (challenge)	9
Total	**74**

Imi: imipenem; Mer: meropenem; Ert: ertapenem

4C- Ciprofloxacin	Total	Grade
resistant	61	4
snnp, ± refer (susceptibility)	3	ungraded
no report on challenge	1	0
snnp, ± refer (challenge)	9	ungraded
Total	**74**	

4D– SXT	Total	Grade
resistant	62	4
snnp, ± refer (susceptibility)	2	ungraded
no report on challenge	1	0
snnp, ± refer (challenge)	9	ungraded
Total	**74**	

4F- Gentamicin	Total
resistant	50
susceptible	11

4G- Tobramycin	Total
resistant	45
intermediate	8

sistant to other carbapenems in automated systems should be re-tested by a true MIC method such as gradient diffusion or broth microdilution. As shown in Table 4E, the majority of laboratories called the isolate ertapenem-resistant. Most laboratories also called imipenem and or meropenem susceptible.

The MICs for both these morphotypes to ertapenem were around 2 - 4 mg/L, but both were susceptible to imipenem and /or meropenem when tested by an alternative method. Because of the variability in observations the results in Table 4E were not graded.

Although it was inadvertent, this sample did in fact have two morphtypes. It is possible to have more than one morphotype of a specific pathogen in a clinical sample. In some cases those may have differing susceptibility patterns, although in this sample both morphotypes harboured ESBLs so that reporting for the cephalosporins should be consistent. In addition both were ertapenem resistant (MIC > 0.5 mg/L) or intermediate. Variability occurred in some other antimicrobials as shown in Table 4.

Laboratories that reported on only one morphotype were not penalized so long as they indicated the strain was ESBL positive and were alert to the possibility of carbapenem resistance because of the ertapenem observation.

Comments on Susceptibility Results

In Table 4A: Piperacillin-Tazobactam. The two laboratories that gave no report on susceptibility were ungraded. Laboratories may have different algorithms for reporting susceptibilities of antimicrobial-resistant organisms. This isolate tested as resistant. It is useful to report the agent to prevent usage in this case. The laboratory that found it susceptible should review their test systems.

Table 4B: Third Generation Cephalosporins. The laboratory that gave no report of susceptibility was graded 0. Since this was an ESBL, the 3rd generation cephalosporins should be considered as resistant, and should be reported as such to ensure that the agent is not used – particularly is they test as resistant. The same was true for "No report of susceptibility" in Tables 4C (trimethoprim-sulfamethoxazole) and 4D (ciprofloxacin).

Table 4E: Carbapenems. These results were not graded but deserve comment. Three laboratories reported ertapenem as Intermediate. Two of these labs obtained MICs >2 (4 and 8) and should have been reported as resistant, the third lab did not report MIC values. Further, testing by another MIC method should would indicate that both morphotypes were both imipenem and meropenem susceptible using the latest guidelines from CLSI. Those two agents may appear to test as falsely resistant in automated susceptibility systems, hence the importance of performing a second method.

Tables 4F and 4G showed that there were a significant number of laboratories that observed different results for gentamicin and tobramycin. Most *K. pneumoniae* if resistant to either agent, are most likely to be resistant to the other because the usual inactivating enzymes confer resistance to both gentamicin and tobramycin. These are not graded.

ANTIMICROBIAL SUSCEPTIBILITY

Resistance to carbapenems can be caused by various mechanisms: production of carbapenemases (carbapenem-specific β-lactamases), efflux pumps, and decreased expression or function of porins and PBPs.

Any combination of these mechanisms can cause high levels of resistance to carbapenems in *Klebsiella pneumoniae* [1].

Carbapenemases are specific β-lactamases with the ability to hydrolyze carbapenems and they appear to be the most widespread cause of carbapenem resistance [2].

Mutations or decreased expression of porins results in decreased entry of carbapenems into the periplasm; these mechanisms exist in, *K. pneumoniae* [3]. Not all carbapenems interact with OMPs the same way; some carbapenems are affected by certain porins more than others (193).

Efflux porins as a mechanism for carbapenem resistance have been reported mostly for *P. aeruginosa* [4] and *E. aerogenes* [5].

Carbapenems are stable to almost all β-lactamases including extended-spectrum β-lactamases (ESBLs) however, Zhanel et al showed that MIC90 for ertapenem in ESBL-producing isolates of *E.coli* and *K. pneumoniae* increased by up to three doubling dilutions relative to the wild strains. MIC90s for imipenem or meropenem did not increase or at most one doubling-dilution increase was observed.

Despite these increases, the ESBL-producing isolates remained susceptible to ertapenem.

Combination of an ESBL along with membrane permeability defects can confer ertapenem resistance in *K. pneumoniae* in the absence of carbapenemases [6].

Fortunately this scenario is relatively uncommon in Canada, but it is prudent to be vigilant. The more recent observations of outbreaks due to OXA-48 carbapenemases in *K. pneumoniae* shows that such strains may have relatively low but still resistant MICS to ertapenem and about two-thirds remain susceptible to imipenem.[7] The *K. peneumoniae* in this challenge did not harbour an OXA-48 enzyme.

CLINICAL RELEVANCE

Multidrug resistant strains of *Enterobacteriaceae* that produce carbapenemases have become a serious threat in some hospitals. In some hospitals in the eastern United States KPC-producing *Klebsiella* have prevalence rates of more than 50%. In deep-seated infections, these organisms cause significant morbidity and mortality, and outbreaks have shown that they can spread readily to other patients with significant consequences. Further, they are difficult to uncover in many surveillance studies of gastrointestinal carriage.

Because these microorganisms usually carry resistance determinants for other antimicrobial classes besides beta-lactam agents, there are few options for treatment. Some strains may have susceptibility to agents such as tigecy-

cline, or colistin, or potentially fosphomycin (in the urinary tract), but there is little else currently available.

It is therefore important to determine quickly if the surrogate ertapenem result is the harbinger of a carbapenemase (with concomitant true resistance to other carbapenems) or is over calling resistance in those other agents which may still be susceptible and effective.

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