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## Surveillance of Gram-negative bacteria: Impact of variation in current European laboratory reporting practice on apparent multidrug resistance prevalence in paediatric bloodstream isolates

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<b>Abstract:</b>	<p><b>Purpose:</b> This study evaluates whether estimated multidrug resistance (MDR) levels are dependent on the design of the surveillance system when using routine microbiological data.</p> <p><b>Methods:</b> We used antimicrobial resistance data from the Antibiotic Resistance and Prescribing in European Children (ARPEC) project. MDR status of bloodstream isolates of <i>Escherichia coli</i>, <i>Klebsiella pneumoniae</i> and <i>Pseudomonas aeruginosa</i> was defined using ECDC-endorsed standardised algorithms (non-susceptible to at least 1 agent in 3 or more antibiotic classes). Assessment of MDR status was based on specified combinations of antibiotic classes reportabed as part of routine surveillance activities. The agreement between MDR status and resistance to specific pathogen-antibiotic class combinations was assessed.</p> <p><b>Results:</b> Based on all available antibiotic susceptibility testing, the proportion of MDR isolates was 31% for <i>E. coli</i>, 30% for <i>K. pneumoniae</i> and 28% for <i>P. aeruginosa</i> isolates. These proportions fell to 9%, 14% and 25%, respectively, when based only on classes collected by current ECDC surveillance methods. Resistance percentages for specific pathogen-antibiotic class combinations were lower compared with MDR percentages, except for <i>P. aeruginosa</i>. Accordingly, MDR detection based on these had low sensitivity for <i>E. coli</i> (2-41%) and <i>K. pneumoniae</i> (21-85%).</p> <p><b>Conclusions:</b> Estimates of MDR percentages for Gram-negative bacteria are strongly influenced by the antibiotic classes reported. When a complete set of results requested by the algorithm is not available, inclusion of classes frequently tested as part of</p>	

	routine clinical care greatly improves detection of MDR. Resistance to individual pathogen-antibiotic class combinations should not be considered reflective of MDR percentages in Enterobacteriaceae.
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1 **Surveillance of Gram-negative bacteria: Impact of variation in current European laboratory**  
2 **reporting practice on apparent multidrug resistance prevalence in paediatric bloodstream**  
3 **isolates**

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26 Keywords: surveillance, Gram negative bacteria, multidrug resistance, routine data

27 **Abstract**

28 Purpose: This study evaluates whether estimated multidrug resistance (MDR) levels are dependent on  
29 the design of the surveillance system when using routine microbiological data.

30 Methods: We used antimicrobial resistance data from the Antibiotic Resistance and Prescribing in  
31 European Children (ARPEC) project. MDR status of bloodstream isolates of *Escherichia coli*,  
32 *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was defined using ECDC-endorsed standardised  
33 algorithms (non-susceptible to at least 1 agent in 3 or more antibiotic classes). Assessment of MDR  
34 status was based on specified combinations of antibiotic classes reported as part of routine  
35 surveillance activities. The agreement between MDR status and resistance to specific pathogen-  
36 antibiotic class combinations was assessed.

37 Results: Based on all available antibiotic susceptibility testing, the proportion of MDR isolates was  
38 31% for *E. coli*, 30% for *K. pneumoniae* and 28% for *P. aeruginosa* isolates. These proportions fell to  
39 9%, 14% and 25%, respectively, when based only on classes collected by current ECDC surveillance  
40 methods. Resistance percentages for specific pathogen-antibiotic class combinations were lower  
41 compared with MDR percentages, except for *P. aeruginosa*. Accordingly, MDR detection based on  
42 these had low sensitivity for *E. coli* (2-41%) and *K. pneumoniae* (21-85%).

43 Conclusions: Estimates of MDR percentages for Gram-negative bacteria are strongly influenced by  
44 the antibiotic classes reported. When a complete set of results requested by the algorithm is not  
45 available, inclusion of classes frequently tested as part of routine clinical care greatly improves  
46 detection of MDR. Resistance to individual pathogen-antibiotic class combinations should not be  
47 considered reflective of MDR percentages in *Enterobacteriaceae*.

48

49 **Introduction**

50 Bacteria resistant to multiple antibiotics have been identified as a major challenge for patient  
51 management and public health [1, 2]. Multidrug resistant Gram-negative bacteria (MDR-GNB) are  
52 considered to be particularly worrying because therapeutic options are limited [3, 4]. Furthermore,  
53 certain MDR-GNB, such as those producing extended-spectrum beta-lactamases or carbapenemases  
54 encoded on plasmids, are of concern due to their potential for interspecies plasmid transfer [5, 6].

55 Large-scale national and international surveillance is an important tool in monitoring MDR-GNB  
56 resistance trends [7]. At present, most surveillance relies on collecting results from traditional  
57 antibiotic susceptibility testing (AST) to track resistance epidemiology, including MDR [8-10]. It is  
58 therefore important that the comparability of isolates identified as MDR by surveillance databases is  
59 established. Standardised algorithms for reporting isolates as MDR were proposed in 2012 by a group  
60 of international experts, but these rely on a large number of antibiotics being included in AST (Table  
61 1) [11]. The selection of antibiotic classes for routine testing continues to be highly variable [12-15].  
62 This potentially presents a major challenge for estimating and comparing MDR-GNB prevalence from  
63 routine data given that individual laboratories may not test all antibiotic classes required.

64 Monitoring of specific pathogen-antibiotic class combinations (PACCs) can be an alternative  
65 surveillance strategy to make best use of available routine data [7, 16-18]. Some PACCs have been  
66 suggested as useful for MDR-GNB assessment based on the recognition of an association in  
67 resistance between different antibiotic classes [19].

68 Using data on neonatal and paediatric GNB isolates obtained from the Antibiotic Resistance and  
69 Prescribing in European Children (ARPEC) project, this study evaluates the degree to which  
70 estimated levels of MDR are dependent on surveillance system design when routine microbiological  
71 data are used.

## 72 **Materials and methods**

### 73 **Data source**

74 The study used data from the ARPEC project, which was co-funded by the European Commission DG  
75 Sanco through the Executive Agency for Health and Consumers [20, 21].

76 ARPEC collected anonymised data on antimicrobial resistance between January 2011 and December  
77 2012 from 19 European laboratories located in 12 different countries, each processing samples for one  
78 paediatric department or hospital. ARPEC requested that participating laboratories reported AST  
79 results for isolates of a specified set of bacterial species, and that, where possible, laboratories report  
80 on specific antibiotics. These included antibiotics required for the European Antimicrobial Resistance  
81 Surveillance Network (EARS-Net) 2010 reporting protocol plus some additional antibiotic categories  
82 (Table 1) [16, 22]. The AST results for each antibiotic tested were reportable as Susceptible/  
83 Intermediate/ Resistant (S/I/R) using breakpoints defined by either

84 (1) European Committee on Antimicrobial Susceptibility Testing (EUCAST),

85 (2) Clinical & Laboratory Standards Institute (CLSI),

86 (3) British Society for Antimicrobial Chemotherapy or

87 (4) Société Française de Microbiologie standards,

88 depending on which standards were used in each country [23-27]. Minimal inhibitory concentrations  
89 of antibiotics were not collected. Duplicate isolates (same species with same antibiogram from the  
90 same patient) identified within 4 weeks of the original isolate were excluded as part of the data  
91 collection protocol.

### 92 **Target bacteria**

93 This study examined MDR patterns for three GNB, namely *Escherichia coli*, *Klebsiella pneumoniae*  
94 and *Pseudomonas aeruginosa*.

## 95 **Interpretation of reported antibiotic susceptibility**

96 Individual antibiotics were grouped into antibiotic classes as defined by the MDR classification  
97 algorithms (Table 1) [11]. Isolates reported as I or R to an antibiotic representative of an antibiotic  
98 class were classified as non-susceptible to that class. In the case of AST results for multiple antibiotics  
99 representative of one class, the isolate was classified as non-susceptible if I or R to any of the  
100 antibiotics tested from that class. Isolates were defined as MDR-GNB if non-susceptible to  $\geq 3$   
101 relevant antibiotic classes [11].

## 102 **Identification of multidrug resistant Gram negative bacterial isolates**

103 The proportion of isolates of each of the three species considered to show multidrug resistance was  
104 then calculated using three sets of antibiotic classes (Table 1):

105 (1) ARPEC set: MDR status was defined by applying the MDR algorithm and based on information  
106 from all classes reported to ARPEC;

107 (2) EARS-Net set: MDR status was defined by applying the MDR algorithm, but based solely on  
108 information for classes included in the EARS-Net protocol;

109 (3) Routine set: MDR status was defined by applying the MDR algorithm, and based on antibiotic  
110 classes with a high level of reported results across all ARPEC laboratories. Classes were included in  
111 this set if AST information was available for at least 85% of isolates. The level of required reporting  
112 was chosen to reflect classes routinely tested for the bacteria of interest in the majority of laboratories.

113 As both the EARS-Net and routinely tested classes are subsets of the ARPEC classes, an isolate  
114 classified as MDR on the basis of the either set was also considered to be MDR based on the ARPEC  
115 set.

## 116 **Evaluation of single pathogen-antibiotic class combinations**

117 It was also assessed whether specific pathogen-antibiotic class combinations (PACCs), suggested to  
118 be critical indicators of MDR by European, US and global professional and/or public health bodies

119 (Table 1), could identify MDR-GNB as detected on the basis of all available data, that is the ARPEC  
120 set [7, 17-19].

121 The specific PACCs of interest were *E. coli* and higher-generation cephalosporins, fluoroquinolones,  
122 aminoglycosides and carbapenems, *K. pneumoniae* and higher-generation cephalosporins and  
123 carbapenems, and *P. aeruginosa* and carbapenems.

124 We defined its sensitivity as the proportion of isolates classified as susceptible for each PACC among  
125 those flagged as MDR from the ARPEC set, and its specificity as the proportion of isolates classified  
126 as non-susceptible for each PACC that were identified as not MDR from the ARPEC set.

### 127 **Statistical analysis**

128 All statistical analyses were carried out using STATA® V12.1, Statacorp, Texas, USA. Whenever  
129 95% confidence intervals (95%CI) are given for proportions, these were calculated by applying an  
130 exact method for binomial data.

131 **Results**

132 In total, 685 isolates were included in the analysis (375 *E. coli*, 176 *K. pneumoniae*, 134 *P.*  
133 *aeruginosa*).

134 **Antibiotic classes included in the Routine set**

135 The classes with reported AST results for the participating centres were very diverse, and there was  
136 no consistent pattern of classes among hospitals located in the same geographical region (data not  
137 shown). No laboratory consistently reported on all classes that were included in the ARPEC protocol.  
138 There was more consistency for the subset of EARS-Net antibiotic classes, with AST results available  
139 for at least 85% of isolates of all three species.

140 There were several classes for which AST testing data were also available for at least 85% of isolates.  
141 The additional frequently tested PACCs included *E. coli* and *K. pneumoniae* AST results for  
142 penicillins/beta-lactamase inhibitor (91% and 96% of isolates), folate pathway inhibitors (86% and  
143 86%) and antipseudomonal penicillins/beta-lactamase inhibitor (85 and 85%). These were then  
144 included in the Routine set (Table 1). The only additional ARPEC antibiotic class relevant for *P.*  
145 *aeruginosa* MDR classification was monobactams, for which AST results were reported for only 47%  
146 of isolates.

147 **Identification of MDR status according to EARS-Net, Routine and ARPEC sets**

148 The proportion of MDR isolates based on the most complete ARPEC set was 30% (95%CI 27-34%)  
149 for all three GNB. Figure 1 shows the number of isolates classified as MDR using the EARS-Net set,  
150 the Routine set and the ARPEC set and the overall proportion estimated as MDR for each pathogen.

151 Table 2 shows the proportion estimated as MDR for each set. Extending the set from the limited  
152 EARS-Net set to the Routine set identified an additional 96 MDR isolates, more than doubling the  
153 estimate of MDR GNB from 13% (95%CI 11-16%) to 27% (95%CI 24-31%). This was most marked  
154 for *E. coli* and *K. pneumoniae* isolates (Figure 1 and Table 2). A similar underestimation on the basis  
155 of the EARS-Net set was not observed for *P. aeruginosa*.

156 For *E. coli* and *K. pneumoniae*, extending assessment to the Routine set meant their MDR  
157 classification was based on three additional ACs (Table 1). Routine set- based MDR status performed  
158 much better than categorization based on the EARS-Net set alone. In contrast, comparing routine and  
159 ARPEC set MDR status, only very few additional isolates were identified as MDR when the more  
160 complete ARPEC set was used.

### 161 **Identification of MDR status based on specific pathogen-drug combinations**

162 The specific PACCs of interest were *E. coli* and higher-generation cephalosporins, fluoroquinolones,  
163 aminoglycosides and carbapenems (reported for 98%, 99%, 98% and 97% of isolates, respectively),  
164 *K. pneumoniae* and higher-generation cephalosporins and carbapenems (reported for 99% and 99% of  
165 isolates, respectively), and *P. aeruginosa* and carbapenems (reported for 98% of isolates).

166 *E. coli* had the following PACC non-susceptibility profiles based on reported AST results: 13%  
167 (95%CI 9-16%) for third/fourth generation cephalosporins, 13% (95%CI 10-18%) for  
168 fluoroquinolones, 13% (95%CI 10-17%) for aminoglycosides and <1% (95%CI 0.1-2%) for  
169 carbapenems. For *K. pneumoniae*, resistance percentages for third/fourth generation cephalosporins  
170 were 32% (95%CI 25-40%) and for carbapenems 6% (95%CI 3-11%). *P. aeruginosa* isolates showed  
171 30% antipseudomonal cephalosporin resistance (95%CI 22-38%) and 31% carbapenem resistance  
172 (95%CI 24-40%). Resistance to higher generation cephalosporins was 21% (95%CI 18-24%) for all  
173 three species. The corresponding resistance percentage for carbapenems was 8% (95%CI 6-11%).

174 Figure 2 displays the number and percentage of isolates that would be appropriately classified as  
175 MDR for each PACC. Isolates are classified as MDR on the basis of the ARPEC set.

176 For *E. coli*, resistance to the specified PACCs failed to correctly identify MDR status for more than  
177 half of the isolates. Aminoglycosides had the best sensitivity (i.e. ability to identify MDR when it was  
178 present) of 41% (Table 3). *E. coli* carbapenem resistance was very rare in the ARPEC dataset, in  
179 contrast to MDR- *E. coli*, and was of very little value in identifying MDR- *E. coli*.

180 For *K. pneumoniae*, both cephalosporin and carbapenem resistance were more strongly associated  
181 with MDR status than for *E. coli* isolates. Third/ fourth generation cephalosporin resistance had a  
182 sensitivity of 85%. However, again carbapenem resistance was not predictive of MDR-*K. pneumoniae*  
183 (sensitivity 21%).

184 For *P. aeruginosa*, both cephalosporin and carbapenem resistance showed a sensitivity of more than  
185 85% for detecting MDR isolates. For all three GNBs, the specificity (the ability to exclude MDR  
186 when it was absent) of the selected PDCs was above 90%. Thus, the rate of false classification of  
187 isolates as not MDR based on absence of resistance to the PACCs reviewed was low.

188 **Discussion**

189 The surveillance definition of multidrug resistance requires the availability of a large number of  
190 susceptibility testing results for correct classification of isolates [11]. If monitoring and comparison of  
191 prevalence of MDR-GNB is to be an aim for on-going surveillance activities collecting routine  
192 microbiology AST data, the optimal strategy for detecting MDR organisms from such data needs to  
193 be established. Current surveillance activities tend to request the AST results for a limited subset of  
194 antibiotic classes listed by the expert MDR classification algorithm [16].

195 In our dataset, the percentage of MDR-GNB isolates was significantly lower (13%) when based on a  
196 more limited set of antibiotic classes, such as that used by EARS-Net, compared with the full set  
197 available (30%). Utilizing the full set of antibiotic classes reportable as part of the ARPEC project, the  
198 proportion of paediatric MDR-*E.coli*, *K. pneumoniae* and *P.aeruginosa* isolates was around 30% and  
199 similar for all three pathogens. Such high levels of isolates with resistance to multiple drugs are  
200 concerning and of interest for tracking the epidemiology of resistant GNB over time.

201 Our study raises several important points regarding the potential of capturing MDR-GNB based on  
202 currently available routine microbiology data purely for surveillance:

203 (1) Routine reporting of AST data by the 19 European laboratories participating in ARPEC only  
204 variably included results for requested antibiotic classes that are part of the classification algorithms  
205 for *E. coli*, *K. pneumoniae* and *P. aeruginosa*. A direct application of the MDR algorithms is therefore  
206 not possible.

207 (2) Limited AST result data also cannot be used to reliably estimate the proportion of MDR-GNB. As  
208 the ARPEC dataset includes only European isolates, the performance of the current European  
209 surveillance system was evaluated. The EARS-Net set of antibiotic classes appeared to lack  
210 sensitivity for detecting MDR-GNB. Inclusion of additional frequently tested and reported antibiotic  
211 classes increased detection of MDR-*E. coli* and *K. pneumoniae* (from 30% detected by the EARS-Net  
212 set to 90% based on the routine set for *E. coli* and from 46% to 92% for *K. pneumoniae*). This was in

213 contrast to *P. aeruginosa*, for which the ARPEC set included only one additional antibiotic class  
214 compared with EARS-Net reporting.

215 (3) A small number of individual PACCs currently represent the typical method for reporting  
216 antimicrobial resistance surveillance internationally (REF). Disappointingly, resistance detected in  
217 individual PACCs was not reliable in detecting MDR isolates. This was especially marked for *E. coli*  
218 isolates, for which resistance to higher generation cephalosporins, for example, had a sensitivity of  
219 only 36% for detecting MDR. *E. coli* is the GNB with the largest number of antibiotic classes in the  
220 MDR classification algorithm and in ARPEC reporting. This may increase detection of many different  
221 resistance combinations, especially if multiple different resistance phenotypes occur.

222 Some of the challenges may be explained by the fact that surveillance collects data primarily  
223 generated to inform clinical decision-making: Approaches to AST are likely to be guided by the need  
224 to optimally inform patient therapy rather than by the need to generate a complete AST dataset for  
225 MDR classification. This type of selective AST based on clinical needs could introduce bias when  
226 these data are interpreted for public health purposes [28]. Bias could be magnified when laboratories  
227 engage in so-called first and second line testing: some antibiotic classes are evaluated only when  
228 resistance to antibiotics included in a first line panel is detected [12].

229 Several limitations need to be considered when interpreting ARPEC data. ARPEC does not cover all  
230 antibiotic classes recommended in the recent expert proposal [11]. It is therefore possible that some  
231 isolates identified as not MDR in ARPEC would in fact be MDR if AST data for all relevant classes  
232 were available. It is also possible that antibiotic classes tested for some of the reported isolates were  
233 suppressed during ARPEC data entry. This seems unlikely, given the relative uniformity of reporting  
234 for each species by each laboratory.

235 The actual percentages of MDR-GNB reported in this study should be interpreted with caution, as  
236 hospitals reporting to ARPEC were tertiary institutions with a patient population not representative of  
237 patients in other inpatient settings and potentially at higher risk of MDR-GNB [20, 21]. Pooling of  
238 data prohibits the identification of any differences between individual participating centres, some of

239 which may have had higher or lower than average MDR-GNB percentages. Finally, the burden of  
240 MDR-GNB cannot be estimated because data are presented as resistance percentages rather than  
241 infection prevalence or incidence [29].

242 All isolates represent neonatal or paediatric blood cultures. The antibiotics used to treat bloodstream  
243 infections in neonates and children may differ from treatment choices for adults. This could be  
244 reflected in the antibiotic classes selected for AST, potentially limiting the transferability of the results  
245 to isolates from adults. However, most laboratories process microbiological samples from both adult  
246 and childhood patients. It is unlikely that AST strategies will be relevantly different for neonatal and  
247 paediatric isolates in these settings.

248 Surveillance of AMR patterns and trends is necessary to target interventions to reduce the selection  
249 and spread of resistant bacteria, and often relies on routine samples collected as part of on-going  
250 clinical care. The limitations and biases associated with the use of routine microbiology data in  
251 surveillance have been widely discussed [8, 28, 29]. Resistance percentages of individual PACCs and  
252 the EARS-Net set currently in use in Europe do not on the whole provide reliable MDR estimates.  
253 This study shows that if MDR surveillance is to be added to the task list of on-going international  
254 surveillance, interpretation of the new algorithm will be limited by the variability in AST strategies in  
255 microbiological laboratories. MDR-GNB detection could be immediately improved by added  
256 surveillance of antibiotic classes already widely tested as part of clinical care. As demonstrated, a  
257 larger percentage of MDR-GNB isolates is likely to be identified with such an approach.

258

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262 data collection or data analysis.

263 **Conflict of interest**

264 JAB's husband is senior corporate counsel at Novartis International AG, Basel, Switzerland, and  
265 holds Novartis stock and stock options. MS chairs and APJ is a member of the Department of Health  
266 Expert Advisory Committee on Antimicrobial Resistance and Healthcare Associated Infection  
267 (ARHAI). All other authors have no conflicts of interest to declare.

268 **Ethical approval and informed consent**

269 The study was assessed against the National Research Ethics Service "Defining Research" leaflet by  
270 the Joint Research Office at the lead centre (St George's University of London, UK) and was found  
271 not to constitute research. The local research ethics committee confirmed that formal evaluation was  
272 not required. Participating centres were instructed to seek local ethical approval if legally required in  
273 their setting and were asked to confirm this at the time they submitted data. Informed consent was not  
274 required as all collected data were fully anonymised, and there was no contact with patients and/or  
275 their families and no interventions or changes to treatment and management were made.

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393

394 Table 1: Summary of the sets of antibiotic classes recommended for detection of MDR-GNB (algorithm) and available from ARPEC and EARS-Net (11, 16).

395 In addition, pathogen antibiotic class combinations (PACCs) used by different surveillance networks are shown (7, 17-19).

Pathogens	<i>E. coli</i>								<i>K. pneumoniae</i>								<i>P. aeruginosa</i> <sup>a</sup>							
	Sets				PACCs				Sets				PACCs				Sets				PACCs			
	MDR algorithm	ARPEC	EARS-Net	Routine	ECDC	WHO	US	UK	MDR algorithm	ARPEC	EARS-Net	Routine	ECDC	WHO	US	UK	MDR algorithm	ARPEC	EARS-Net	Routine	ECDC	WHO	US <sup>b</sup>	UK
<b>Antibiotic classes</b>																								
Aminoglycosides	X	X	X	X				X	X	X	X	X					X	X	X	X				
Anti-MRSA cephalosporins	X								X															
Anti-pseudomonal penicillins plus beta-lactamase inhibitor	X	X		X					X	X		X					X	X	X	X				
Carbapenems	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Non-extended spectrum cephalosporins (1 <sup>st</sup> & 2 <sup>nd</sup> gen.)	X	X							X	X														
Extended spectrum cephalosporins (3 <sup>rd</sup> & higher gen.)	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X				X
Cephamycins	X	X							X	X														
Fluoroquinolones	X	X	X	X		X		X	X	X	X	X					X	X	X	X				
Folate pathway inhibitors	X	X		X					X	X		X												
Glycylcyclines	X								X															
Monobactams	X	X							X	X							X	X						
Penicillins (Ampicillin)	X	X	X	X																				
Penicillins plus beta-lactamase inhibitor	X	X		X					X	X		X												
Phenicols	X	X							X	X														
Phosphonic acids	X								X								X							
Polymyxins	X								X								X							
Tetracyclines	X	X							X	X														
Number of antibiotic classes included in sets used to calculate % of MDR-GNB isolates	17	13	5	8	-	-	-	-	16	12	4	7	-	-	-	-	8	6	5	5	-	-	-	-

396

397

398 <sup>a</sup>For *P. aeruginosa*, all antibiotic classes only include antibiotics with antipseudomonal activity.

399 <sup>b</sup>Note that *P. aeruginosa* is not included in the US National Healthcare Safety Network surveillance.

400 Table 2: MDR-GNB percentages based on EARS-Net, Routine and ARPEC sets (see Table 1 for  
 401 definition of sets).

	Total n isolates	MDR isolates		
		% MDR based on EARS-Net set (95%CI)	% MDR based on Routine set (95%CI)	% MDR based on full ARPEC set (95%CI)
<i>E. coli</i>	375	9.3 (6.6-12.7)	28.5 (24.0-33.4)	31.2 (26.5-36.2)
<i>K. pneumoniae</i>	176	13.6 (8.9-19.6)	27.3 (20.8-34.5)	29.6 (22.9-36.9)
<i>P. aeruginosa</i>	134	24.6 (17.6-32.8)	n/a	28.4 (20.9-36.8)
<b>All GNB</b>	685	13.4 (11.0-16.2)	27.4 (24.1-31.0)	30.2 (26.8-33.8)

402

403

404 Table 3: Detection of MDR-GNB when specific pathogen-antibiotic class combination antimicrobial  
 405 susceptibility testing results are assumed to represent MDR status. The percentage of isolates  
 406 misclassified as MDR or not MDR based on PACC results is compared with MDR based on all  
 407 ARPEC antibiotic categories (see Table 1).

		MDR classification			
		n MDR correctly identified	Sensitivity of PACC in % (95%CI)	n not MDR correctly identified	Specificity of PACC in % (95%CI)
<i>E. coli</i>	3 <sup>rd</sup> /4 <sup>th</sup> generation cephalosporins	41/114	36.0 (27.2-45.5)	254/259	98.1 (95.6-99.4)
	Fluoroquinolones	46/115	40.0 (31.0-49.6)	255/258	98.8 (96.6-99.8)
	Aminoglycosides	48/116	41.4 (32.3-50.9)	253/259	97.7 (95.0-99.1)
	Carbapenems	2/117	1.7 (0.2-6.0)	245/245	100.0 (98.5-100.0)
<i>K. pneumoniae</i>	3 <sup>rd</sup> /4 <sup>th</sup> generation cephalosporins	44/52	84.6 (71.9-93.1)	123/135	91.1 (85.0-95.3)
	Carbapenems	11/52	21.2 (11.1-34.7)	122/122	100.0 (97.0-100.0)
<i>P. aeruginosa</i>	Antipseudomonal cephalosporins	34/38	89.5 (75.2-97.1)	96/102	94.1 (87.6-97.8)
	Carbapenems	33/38	86.8 (71.9-95.6)	96/105	91.4 (84.4-96.0)

408

409

410 Figure 1: Number and percentage of isolates classified as MDR based on different sets of antibiotic  
411 classes (see Table 1 for definition of sets). The total number of isolates for each bacterial species is  
412 shown at the top of the bar.

413

414 Figure 2: Number and percentage of isolates identified correctly or incorrectly as MDR based on  
415 individual pathogen-antibiotic class combinations (PACCs). White stacks correspond to isolates  
416 neither resistant to the PACC nor identified as MDR on the basis of the ARPEC set (see Table 1 for  
417 definition). The total number of isolates for each bacterial species are shown underneath.

418

419 3/4GC: third or fourth generation cephalosporin, QUIN: fluoroquinolone, AMG: aminoglycoside,  
420 CPM: carbapenem. For *P. aeruginosa*, only cephalosporins with antipseudomonal activity were  
421 considered.

Figure 1

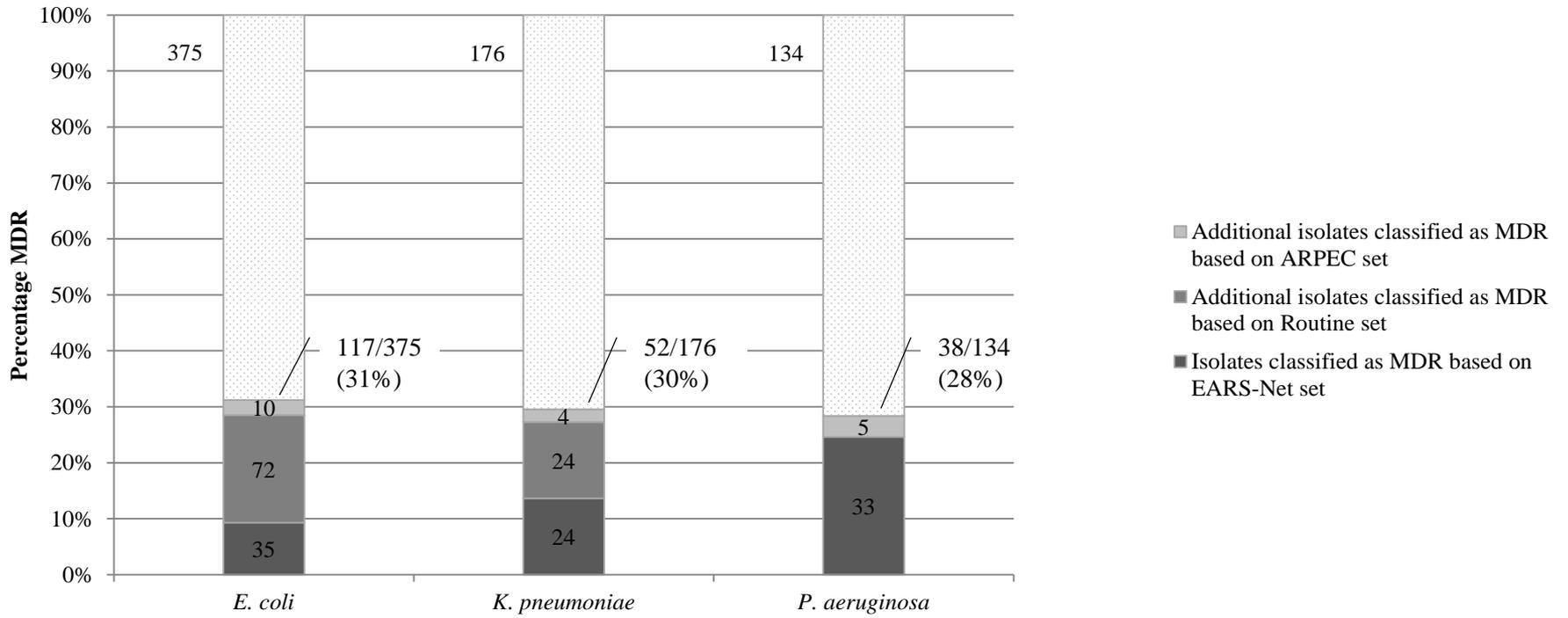


Figure 2

