

## Trends in antibiotic resistance over time among pathogens from Canadian hospitals: results of the CANWARD study 2007–11

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**Objectives:** Antimicrobial resistance patterns change over time and longitudinal surveillance studies provide insight into these trends. We sought to describe the important trends in antimicrobial resistance in key pathogens across Canada to provide useful information to clinicians, policy makers and industry, to assist in optimizing antimicrobial therapy, formulary choices and drug development.

**Methods:** We analysed longitudinal data from the CANWARD study using a multivariate regression model to control for possible effects of patient demographics on resistance, in order to assess the impact of time on antimicrobial resistance independent of other measured variables.

**Results:** We identified several key trends in common pathogens. In particular, we observed a statistically significant increase in the proportion of *Escherichia coli* isolates that were resistant to extended-spectrum cephalosporins and fluoroquinolones, an increase in the proportion of *Klebsiella pneumoniae* isolates that were resistant to extended-spectrum cephalosporins, a reduction in the proportion of *Staphylococcus aureus* that were methicillin, clindamycin and trimethoprim/sulfamethoxazole resistant, and a reduction in the proportion of *Pseudomonas aeruginosa* that were fluoroquinolone and gentamicin resistant.

**Conclusions:** Although some of these trends, such as the dramatic increase in fluoroquinolone and cephalosporin resistance in *E. coli*, can be attributed to the emergence and global spread of resistant clones (e.g. ST131 *E. coli*), others remain unexplained. However, recognizing these trends remains important to guide changes in empirical antimicrobial therapy and drug development.

**Keywords:** resistance, antimicrobial, *Escherichia*, *Staphylococcus*, *Pseudomonas*

### Introduction

Microbiologists and clinicians have long recognized that patterns of resistance in key pathogens change over time.<sup>1</sup> Although slow emergence of resistance to antimicrobials is the most commonly observed pattern, the re-emergence of susceptible isolates has been observed in certain settings.<sup>1–4</sup> The reasons for changes in antimicrobial susceptibility are multifactorial and although a primary driving force is the use and abuse of antimicrobial agents, other factors, including clonal spread, density of population, human movement, organism fitness, vaccination, pathogenicity

and colonizing ability, all play roles.<sup>5–7</sup> A recent key example has been the rapid emergence of extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* in the community, primarily due to the relative fitness and pathogenicity of the ST131 clone combined with the increasing ease with which these pathogens can be transported long distances in their human hosts.<sup>8–11</sup> Monitoring the emergence of resistance to key antimicrobials over time continues to be an important activity for clinicians and microbiologists, as these trends guide empirical antimicrobial therapy for common infections. Furthermore, monitoring trends also allows the identification of key areas for antimicrobial development, as evidenced by

the recent surge in the early development of anti-Gram-negative agents, ostensibly driven by the emergence of ESBL- and carbapenemase-producing Enterobacteriaceae.<sup>1,12</sup> Such examples include plazomicin, ceftazidime/avibactam, boron-containing  $\beta$ -lactamase inhibitors (e.g. GSK2251052 and RPX7009), iclaprim, monobactam derivatives (e.g. BAL30072) and new carbapenems (e.g. faropenem and biapenem). Unfortunately, few of these are available for clinical use at this time, leading to a paucity of agents available for multidrug-resistant Gram-negative infections (www.bsac.org.uk).

Over the past 5 years, the CANWARD study<sup>13</sup> has observed the antimicrobial resistance patterns of key pathogens in hospital wards, intensive care units (ICUs), emergency wards and hospital-affiliated outpatient clinics. Participating hospitals also collected key demographic characteristics of patients submitting samples, allowing for in-depth statistical analysis, potentially identifying valuable trends in resistance over time. The primary purpose of this study was to use multiple regression analysis to describe significant trends independent of other demographic variables collected in the study.

## Materials and methods

### Bacterial isolates

Clinically relevant bacterial isolates were collected from hospital sites across Canada between 2007 and 2011. Between 10 and 15 hospital sites from eight provinces across Canada were involved in the study each year. Isolates were collected from medical and surgical wards, ICUs, emergency rooms and hospital-affiliated outpatient clinics. A total of 27123 isolates were collected during the study; however, for the purpose of this analysis, only *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Enterococcus faecium* isolates were analysed. Additional information collected for each specimen included patient age and gender, specimen type (cutaneous, urine, respiratory or blood) and patient location at the time of collection (outpatient, inpatient or ICU settings). Data from all participating hospitals were pooled to reflect the Canadian situation. Details on the study hospital locations and specimens can be found in Zhanel et al.<sup>13</sup>

The CANWARD study receives annual approval by the University of Manitoba Research Ethics Board (H2009:059).

### Antimicrobial susceptibility testing

Susceptibility testing was performed using broth microdilution panels following methodology and quality control recommendations outlined in CLSI document M07-A9.<sup>14</sup> Susceptibility was interpreted using CLSI breakpoints in the M100-S21 document.<sup>15</sup> ESBL-producing *E. coli* and *K. pneumoniae* were screened and confirmed using the criteria described in CLSI document M100-S21.<sup>15</sup> All confirmed ESBL-producing isolates were subsequently genetically characterized by sequencing of  $\beta$ -lactamase genes, as described by Denisuk et al.<sup>16</sup>

### Statistical analysis

Each species was analysed separately. For the purpose of statistical analysis, isolates were defined to be either susceptible or resistant to an antimicrobial. The isolates with intermediate susceptibility were included with the resistant group. The variables included year of study, patient age (<18 years, 18–65 years or >65 years), gender (male or female), patient location (ICU, hospital inpatient or outpatient) and specimen type (blood, urine, cutaneous or respiratory). A second-degree factorial

multivariate logistic regression analysis using available demographic variables was used to isolate the effect of time on susceptibility from changes in demographic variables that may have occurred in the dataset as well as second-degree interactions among the variables. Initially, a stepwise regression model was created including all the variables and second-degree interactions. In order to exclude variables without significant impact on the model, variables were excluded from the final model in such a way to minimize the Akaike information criterion. Finally, a regression model was created including only the variables and interactions with a significant impact on resistance to an antimicrobial. *P* values  $\leq 0.05$  were considered significant. Statistical analysis was performed using JMP 10 software (SAS, Cary, NC, USA).

## Results

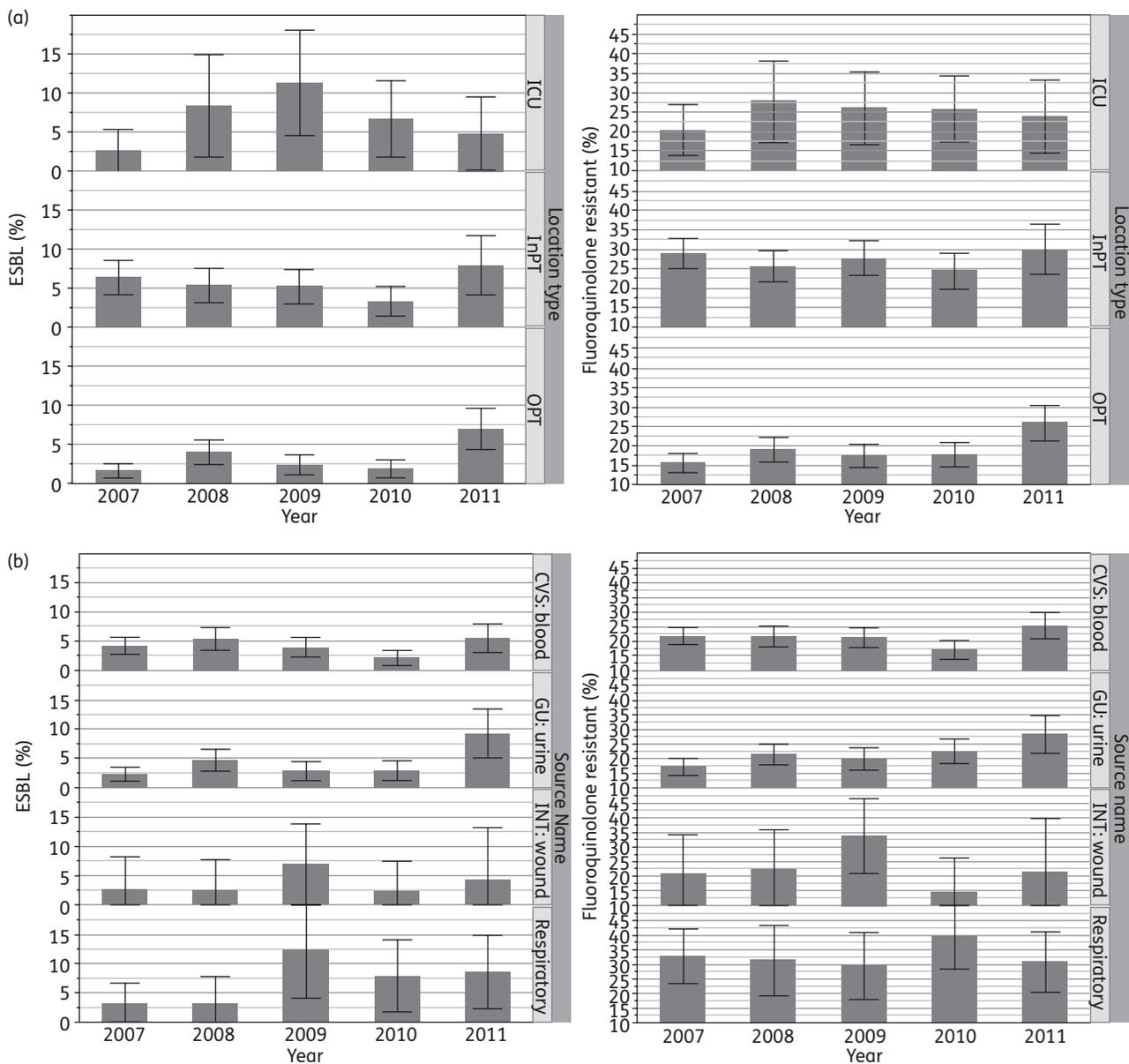
### *E. coli*

There were 5451 *E. coli* isolates collected as part of CANWARD, of which 5396 had complete datasets and were included in the analysis. Overall, multivariate analysis showed that the proportion of isolates producing ESBLs increased significantly over the study period ( $P=0.002$ ) (Table 1). Further analysis demonstrated that the increase was significant in outpatients (1.8%–7.1%,  $P=0.003$ ) but not in other patient types (Figure 1a) and was significant in urine specimens (2.4%–9.4%,  $P=0.008$ ) but not in other specimen types (Figure 1b). Ciprofloxacin resistance also increased over time ( $P=0.017$ ). The increase was largely driven by increased resistance rates over time in urine specimens ( $P=0.002$ ) and in outpatients ( $P=0.001$ ). The increasing proportion of both fluoroquinolone-resistant and ESBL-producing *E. coli* was most pronounced in the period between 2010 and 2011. The proportion of isolates resistant to gentamicin (range 8.2%–11.8%), trimethoprim/sulfamethoxazole (range 26.4%–29.3%), carbapenems (0% in all years) and piperacillin/tazobactam (range 0.5%–1.5%) did not significantly change over time (data not shown).

**Table 1.** Trends in antimicrobial resistance over time observed in the multivariate model

	2007	2008	2009	2010	2011	<i>P</i> value
<i>E. coli</i> (n=5396)						
ESBL positive (%)	3.5	4.9	4.2	2.9	7.1	0.002
ciprofloxacin resistant (%)	20.7	22.3	22.1	20.8	27.0	0.017
<i>S. aureus</i> (n=5437)						
MRSA (%)	26.1	27.0	21.0	21.2	19.3	<0.001
clindamycin resistant (%)	22.8	20.5	16.0	15.5	10.6	<0.001
SXT resistant (%)	3.7	3.7	1.3	1.1	0.4	<0.001
<i>P. aeruginosa</i> (n=2181)						
ciprofloxacin resistant (%)	34.0	24.7	28.7	26.3	21.8	<0.001
gentamicin resistant (%)	39.7	29.0	25.5	24.7	11.8	<0.001
<i>K. pneumoniae</i> (n=1657)						
ESBL positive (%)	1.6	3.3	3.4	3.3	4.0	0.006
<i>S. pneumoniae</i> (n=1782)						
penicillin MIC $\geq 0.12$ mg/L	20.8	19.4	12.0	16.0	16.8	0.015

SXT, trimethoprim/sulfamethoxazole. Only significant trends are shown.



**Figure 1.** (a) Trends in ESBL and fluoroquinolone resistance rates in *E. coli* rates by patient type. Bars represent 95% CI. OPT, outpatient; InPT, inpatient; ICU, intensive care unit patient. (b) Trends in ESBL and fluoroquinolone resistance rates in *E. coli* rates by specimen type. CVS: blood, blood culture isolate; GU: urine, urine culture isolate; INT: wound, wound isolate; Respiratory, respiratory tract isolate. Error bars represent 95% CI.

### *S. aureus*

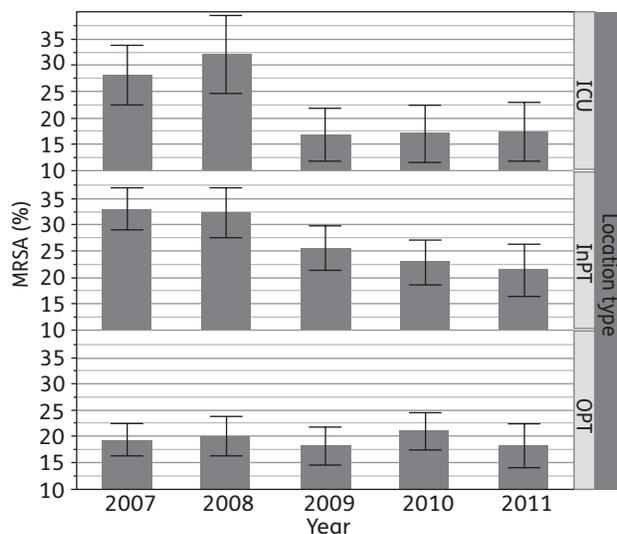
Between 2007 and 2011, 5443 *S. aureus* isolates were collected, of which 5437 had complete datasets and were included in the analysis. The proportion of methicillin-resistant *S. aureus* (MRSA) declined over time ( $P < 0.001$ ) (Table 1). The decline was significant in the inpatient (33.0%–21.2%,  $P < 0.001$ ) and ICU (28.2%–17.7%,  $P < 0.001$ ) populations, while no decline was observed in outpatient populations (Figure 2). The prevalence of clindamycin- and trimethoprim/sulfamethoxazole-resistant strains declined over time ( $P < 0.001$ ) (Table 1). There has been no change over time in isolates with resistance to macrolides (range 34.8%–43.2%) or reduced susceptibility to vancomycin (MIC  $\geq 2$  mg/L) (range 0.2%–1.4% of isolates) (data not shown).

### *P. aeruginosa*

There were 2183 *P. aeruginosa* isolates collected as part of CANWARD, of which 2181 had complete datasets and were included in the analysis. Resistance to ciprofloxacin and gentamicin declined over time ( $P < 0.001$ ) (Table 1). The declines were observed across all specimen types. No significant changes occurred over time in resistance to ceftazidime (range 9.1%–12.8%), piperacillin/tazobactam (range 5.6%–9.1%) or meropenem (range 9.6%–12.0%) (data not shown).

### *K. pneumoniae*

As part of CANWARD, 1659 *K. pneumoniae* isolates were collected, of which 1657 had complete datasets and were included



**Figure 2.** Trends in MRSA rates by patient type. Shaded areas represent 95% CI of slope. OPT, outpatient; InPT, inpatient; ICU, intensive care unit patient. Error bars represent 95% CI.

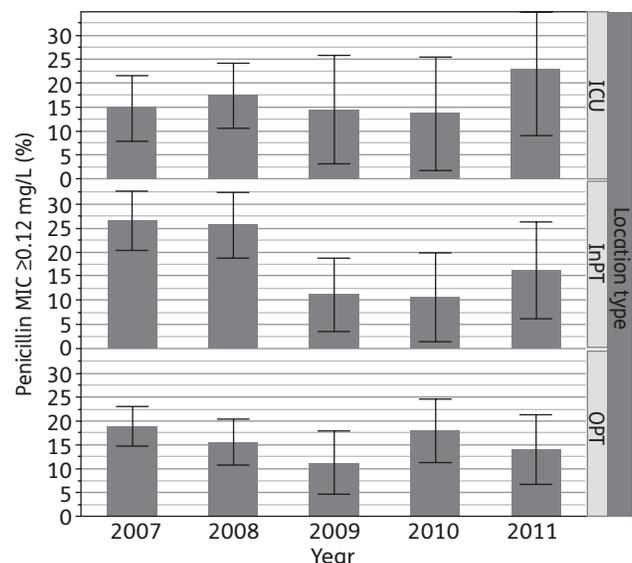
in the analysis. Overall, multivariate analysis showed that the prevalence of ESBL-producing isolates significantly increased over the study period ( $P=0.006$ ) (Table 1). Analysis by patient type showed that a marginally significant ( $P=0.05$ ) increase in ESBL rates was observed in the outpatient population. A similar trend was observed in the ICU population, but it did not achieve significance. Resistance rates to ciprofloxacin (range 4.9%–7.3%), gentamicin (range 1.8%–3.3%), trimethoprim/sulfamethoxazole (range 7.9%–11.1%), meropenem (range 0%–0.3%) and piperacillin/tazobactam (range 1.3%–2.5%) did not change significantly over time (data not shown).

### *E. faecium*

Between 2007 and 2011, 271 *E. faecium* isolates were collected, of which 268 could be included in the analysis. Vancomycin resistance rates ranged from 13.6% to 32% in the 5 year study period. No statistically significant trends were observed over the 5 years of study (data not shown).

### *S. pneumoniae*

There were 1881 *S. pneumoniae* isolates collected as part of CANWARD, of which 1782 were considered in this analysis. Isolates that could not be analysed occurred randomly during the study, primarily due to loss of viability during transport. A small, statistically significant reduction in low-level penicillin resistance (MIC  $\geq 0.12$  mg/L) was noted over time ( $P=0.015$ ). Further analysis demonstrated that the reduction was most significant in inpatients (26.7%–16.4%,  $P=0.003$ ) (Figure 3). No statistically significant changes over time were observed in the multivariate model for resistance to trimethoprim/sulfamethoxazole (range 6.7%–12.4%), levofloxacin (range 0%–1.2%), doxycycline (range 0%–5.1%), clindamycin (range 1.0%–10.3%), clarithromycin (12.0%–22.7%), ceftriaxone (range 0%–0.5%)



**Figure 3.** Trends in *S. pneumoniae* with penicillin MIC  $\geq 0.12$  mg/L by patient type. Error bars represent 95% CI. OPT, outpatient; InPT, inpatient; ICU, intensive care unit patient.

or to high-level resistance to penicillin (MIC  $\geq 2$  mg/L) (range 2.4%–5.0%).

## Discussion

A number of significant trends were observed over the 5 years of this study. The most important of these was the increase in fluoroquinolone-resistant and ESBL-producing *E. coli* and the decline in MRSA and fluoroquinolone-resistant *P. aeruginosa*. The trend for increasing prevalence of ESBL-producing *E. coli* has been described by others in multiple regions of the world and most likely represents the expansion of ESBL-producing clones globally.<sup>17–21</sup> *E. coli* ST131 frequently harbouring the CTX-M-15 ESBL as well as multiple determinants of fluoroquinolone resistance [e.g. *gyrA* and *parC* mutations and *aac(6′)-Ib-cr* and *qnr* genes] has been shown to be a virulent and successful clone, primarily associated with urinary tract infection (UTI) in the community setting.<sup>8,11,18–22</sup> In keeping with the global expansion of this clone, our data demonstrate a significant increase in fluoroquinolone-resistant and ESBL-producing isolates from outpatient urine samples. Indeed, our data demonstrate that the majority of the ESBL-producing isolates belong to ST131.<sup>23</sup> The rapid increase in the proportion of ESBL-producing *E. coli* in the later part of the study could be explained by this clone crossing a critical prevalence threshold subsequently leading to rapid transmission in the population, or alternatively to the acquisition of genetic factors facilitating the transmission and spread of this pathogen. The recent increase in ESBL-producing *E. coli* was noted in six of the eight Canadian provinces studied (data not shown), suggesting that the phenomenon is widespread and not the result of a single area experiencing increasing prevalence. The expansion of the ST131 clone also probably accounts for the increase in fluoroquinolone-resistant isolates, as fluoroquinolone resistance has been shown to occur with a relatively high prevalence among global isolates

of ST131, regardless of the additional presence of the CTX-M enzymes conferring resistance to extended-spectrum cephalosporins.<sup>24</sup> The relative success of this clone as a uropathogen that has been shown to possess a number of uropathogenic virulence factors accounts for the noted increase of both ESBL producers and fluoroquinolone resistance over time in community-associated UTIs seen in this study.<sup>8,20–22</sup> Furthermore, an additional mechanism by which both ESBL-producing and fluoroquinolone-resistant *E. coli* have probably increased in prevalence is the conjugative spread of plasmids encoding both resistance determinants, which has been described worldwide.<sup>7,25</sup>

An unexpected finding in this study was the observed reduction in MRSA over time, particularly in inpatient and ICU populations. Although overall rates declined, no significant decline was observed in the outpatient setting. A number of explanations potentially account for this observation, the simplest being that rigorous infection control practices implemented over the past 5 years have had an impact on the in-hospital acquisition of MRSA.<sup>1</sup> Although this explanation may be partially responsible, no significant changes in infection prevention and control practices have been recommended in Canada in the past decade. An alternative possibility is that the replacement of previously common healthcare-associated clones of MRSA [e.g. Canadian-MRSA type-1 (CMRSA-1) (USA600) and CMRSA-2 (USA100/800)] with community-associated strains [e.g. CMRSA-7 (USA400) and CMRSA-10 (USA300)] has resulted in a reduction of healthcare-associated transmission. This could occur due to the predilection for such strains towards a different demographic, namely antimicrobial-naïve, generally healthy individuals residing in the community, thereby reducing the number of colonized individuals in-hospital.<sup>7</sup> This is likely to result in a reduction of the number of patients colonized in hospitals, therefore reducing the risk of transmission.<sup>7</sup> It is also possible that healthcare-adapted MRSA strains are being replaced by healthcare-adapted non-MRSA strains as hospitals shift selective pressures away from  $\beta$ -lactam therapy for staphylococcal infections. There is less interest in the systematic typing of non-MRSA *S. aureus*, but other centres have reported similar declines in MRSA rates, largely attributable to shifts in epidemic strains.<sup>4,26–29</sup> In some instances, such declines were observed prior to infection control interventions, further supporting the notion that epidemic shifts rather than infection control measures are responsible for declining MRSA rates in many parts of the world.<sup>1</sup> An additional explanation may be the fitness cost associated with multiple resistance determinants, which has been suggested as one of the causes of the decline in healthcare-associated MRSA strains.<sup>29</sup>

Equally unexpected was the decline in fluoroquinolone and gentamicin resistance in *P. aeruginosa*, independent of patient location and specimen type. Regional analysis showed that the reduction in gentamicin resistance was observed in all regions sampled for which data were available for the 5 years (data not shown). However, although most provinces showed some decline in resistance to fluoroquinolone and the decline was significant overall, no particular province on its own achieved a statistically significant decline (or increase) in fluoroquinolone resistance (data not shown). Although the cause of this decline is not clear, similar observations have been made by others, who also reported a decline in fluoroquinolone and gentamicin

resistance between 2001 and 2009.<sup>30</sup> Given the propensity of *P. aeruginosa* to develop resistance rapidly in the context of selective pressure, it is possible that a reduction in fluoroquinolone and gentamicin use in the populations at risk for *Pseudomonas* infections has resulted in a reversal of resistance rates.<sup>31</sup> Although fluoroquinolone consumption in the community setting has not changed significantly over the period of this study ([www.can-r.ca](http://www.can-r.ca)), community usage data probably do not reflect usage among individuals at risk of *Pseudomonas* infections, since most of these are healthcare associated and represent a small proportion of the consumers of fluoroquinolones. Aminoglycosides, in contrast, have generally fallen out of favour in most hospitals, with drugs having fewer associated adverse events being preferred, which probably accounts for increasing susceptibility in *P. aeruginosa*.<sup>32</sup>

Although not as striking as the recent rise in ESBL rates among *E. coli*, we also noted a significant rise in ESBL-producing *K. pneumoniae* during the study period. As with *E. coli*, this trend was also significant in the outpatient population, but no change over time was noted by specimen type. Although similar factors may be driving the expansion of ESBLs in *Klebsiella* as in *E. coli*, there is little evidence to support a clonal expansion of *K. pneumoniae* in Canada at this time. However, others have reported the clonal spread of *K. pneumoniae* ST11, ST15, ST16, ST147 and others in a variety of countries and settings.<sup>18,33,34</sup> Additionally, the conjugative spread of CTX-M-15 plasmids has also been described as a cause of increasing ESBL prevalence in *K. pneumoniae*, which may also be occurring in this surveillance sample.<sup>34</sup> Indeed, characterization of the ESBLs in these isolates demonstrated that 50% were CTX-M-15 producers (data not shown). Further characterization of sequence types and plasmids may demonstrate the mechanisms by which prevalence has increased.

No significant changes were noted in the proportion of vancomycin-resistant enterococci (VRE) found among the *E. faecium* isolates in our multivariate analysis. This is despite a large increase in VRE colonization across Canada over the past decade (<http://www.phac-aspc.gc.ca/nois-sinp/projects/index-eng.php>). The low pathogenicity of VRE is probably responsible for the lack of significant change in VRE prevalence in our study, which includes only isolates causing infections. As VRE prevalence continues to rise, surveillance may demonstrate increases in clinical infections caused by VRE.

The proportion of *S. pneumoniae* with low-level resistance to penicillin (MIC  $\geq 0.12$  mg/L) decreased slightly over the study period. This trend was strongest in the inpatient population. A possible explanation for this trend is the increased uptake of conjugated pneumococcal vaccines among children, which has been shown to result in reduced prevalence of nasopharyngeal colonization and infection by resistant strains covered by the vaccine in both children and adults.<sup>2,35,36</sup> It remains to be seen if the recent emergence of resistant non-vaccine serotypes (e.g. 19A previously not included in the conjugate vaccine) has an impact on clinical disease caused by resistant serotypes.

In conclusion, although this study revealed a number of trends occurring in antimicrobial resistance over time, the most worrisome is the dramatic and consistent increase in fluoroquinolone and extended-spectrum cephalosporin resistance in *E. coli*. The consistency of this observation across Canada and the world confirms that this is a real trend not influenced

by the sampling bias frequently encountered in large-scale surveillance studies. This alarming trend signals an urgent need to focus drug development efforts on agents active against these multidrug-resistant Gram-negative organisms, particularly the development of orally bioavailable agents effective for the treatment of community-associated UTIs.

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CANWARD data can also be found at [www.can-r.ca](http://www.can-r.ca), the official web site of the Canadian Antimicrobial Resistance Alliance (CARA).

## Members of the Canadian Antimicrobial Resistance Alliance (CARA)

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