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Environmental cephalosporin-resistant gram-negative bacteria: what is lurking beyond the hospital doors?

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Abstract

Antibiotic resistant Gram-negative bacilli are being increasingly recognized in community-onset infections. Concerns have been raised regarding environmental surfaces as reservoirs for multidrug-resistant pathogens; in particular, community wastewater samples have been found to harbor cephalosporin- and carbapenem-resistant Enterobacterales. In this report, we used selective media to culture cephalosporin-resistant Gram-negative bacilli from high-touch surfaces in the environment surrounding hospitals in New York City. Of the 336 surfaces swabbed, 66 grew cephalosporin-resistant Gram-negative bacteria. No Enterobacterales were isolated. The most common isolate was *Brucella* (*Ochrobactrum*) spp., accounting for 26 of the 66 positive cultures; 19 originated from surfaces near two hospitals. One isolate of *Brucella* (*Ochrobactrum*) spp. was found to possess $bla_{\text{OXA-24}}$. *Acinetobacter* spp. accounted for 12 of the 66 positive cultures. Forty cultures obtained with saline-saturated gauze were obtained at public transportation centers near hospitals; a comparable 40 cultures were obtained at transportation centers distant from hospitals. Of the 38 positive cultures from transportation surfaces near medical centers, 15 grew *Brucella* (*Ochrobactrum*) spp. In comparison, *Brucella* (*Ochrobactrum*) spp. accounted for only five of the 33 positive transportation cultures distant from medical centers (P = 0.03). Again, none of the cultures grew Enterobacterales. It is reassuring that dried environmental surfaces around hospitals did not contain resistant Enterobacterales. However, *Brucella* (*Ochrobactrum*) spp. was widespread and territorial and may be a reservoir for OXA-type carbapenemases.

Keywords: *environmental contamination; Brucella; Ochrobactrum; Acinetobacter, carbapenem resistance*

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Infections with multidrug-resistant Gram-negative bacteria are associated with poor outcomes and increased expenditures. In 2019, the Centers for Disease Control and Prevention identified carbapenem-resistant *A. baumannii* and Enterobacterales as 'urgent threats' and extended spectrum beta-lactamase (ESBL)-possessing Enterobacterales and multidrug-resistant *P. aeruginosa* as 'serious threats' (1).

Community-onset infections with ESBL Enterobacterales and carbapenem resistant bacteria are being increasingly recognized. ESBL-Enterobacterales have been recovered in water samples and animals; poor sanitation and overcrowding conditions contribute to community-onset infections with these pathogens (2, 3). Community onset cases of carbapenem-resistant Enterobacterales are also recognized. The finding of carbapenemase-producing Enterobacterales in water samples, including wastewater near hospitals, raises concern for community acquisition via environmental contamination (4–6).

Environmental contamination with multidrug-resistant non-fermenting Gram-negative pathogens has also been well documented. In particular, *Acinetobacter* spp. is well equipped to survive prolonged periods in arid conditions (7–9), and outbreaks of infections can follow natural disasters and conflicts (10). Similarly, both *Pseudomonas* spp. and *Stenotrophomonas maltophilia* can survive extended intervals in desiccated environments and are important multidrug-resistant pathogens (11, 12).

In this report, we searched for the presence of cephalosporin-resistant Gram-negative bacilli on environmental surfaces surrounding acute care medical centers in New York City.

Materials and methods

Two datasets of environmental cultures were gathered during July and August 2023. The first dataset involved swabbing high-touch environmental surfaces in areas near (generally within a two-block distance) of eight major

medical centers located in the boroughs of Brooklyn, Manhattan, and Queens of New York City. Most cultures were from outside locations; a few cultures from inside the hospital lobby were permitted. Forty-two sterile rayon-tipped swabs (Puritan Medical Products, Guilford, ME) were first saturated in sterile saline. The environmental surface culture was obtained and inoculated into ~ 2 mLs of MacConkey broth containing 4 μg/mL of ceftazidime and 4 μg/mL of amphotericin B. A 43rd swab served as a sterility control.

The second dataset involved environmental surfaces involving the eight public transportation centers nearby the medical centers; in addition, comparative environmental cultures from eight transportation centers (located in Brooklyn, Long Island, and New Jersey) distant from acute care medical centers were gathered. Five sterile gauze 2X2 inch pads were first saturated with normal saline. High touch areas outside of the transportation center were wiped with the gauze, and the gauze placed in ~ 20 mL of MacConkey broth containing ceftazidime and amphotericin B.

All cultures were incubated at 37°C for up to 48 h. All samples with suspected growth were subcultured on MacConkey agar. Individual colonies were isolated, and susceptibility testing involving ceftazidime and meropenem was performed by the broth microdilution method using cation-supplemented Mueller-Hinton broth (13); *P. aeruginosa* ATCC 27853 was used as a control. Isolates confirmed with ceftazidime MICs ≥ 8 μg/mL (non-susceptible for Enterobacterales by CLSI definitions) were identified by sequencing a fragment of the 16S rRNA gene using previously described primers and PCR conditions (14). Isolates were considered resistant to meropenem if the MIC was ≥ 4 μg/mL. The presence of the β-lactamases TEM, SHV, CTX-M, KPC, NDM, VIM, IMP, and OXA-type carbapenemases was screened using previously described PCR primers and conditions (15).

The survivability of different bacterial isolates in a desiccated environment was also examined. Bacterial isolates were grown to the late-log phase of growth in Mueller-Hinton broth. Cultures were vortexed, and 20 μL aliquots was placed in a sterile 96-well plate. The samples were allowed to dry, and the pellet was resuspended in 100 μL sterile saline at T = 0 (baseline), 3, and 7 days. Serial 1:10 dilutions of the suspension were subcultured on Mueller-Hinton agar plates and incubated overnight; the number of surviving bacteria in the pellet was then determined.

Results

Cultures from environmental surfaces near hospitals

There were 66 cephalosporin-resistant Gram-negative organisms identified from the 336 swab cultures from environmental surfaces near medical centers (Table 1).

Table 1. Ceftazidime-resistant Gram-negative bacilli recovered in environmental swab samples near major medical centers in the New York City

Isolate	Number (% resistant to meropenem)
Achromobacter spp.	3 (33)
Acinetobacter spp.	12 (8)
Brevundimonas spp.	7 (57)
Brucella (*Ochrobactrum*) spp.	26 (4)
Cupriavidus spp.	5 (100)
Pseudomonas spp.	3 (33)
Rhizobium/Agrobacterium spp.	6 (17)
Stenotrophomonas spp.	1 (100)
Other*	3 (33)

*Halotalea spp. (n = 1) and Pantoea spp. (n = 2).

Among the 12 *Acinetobacter* spp., 16S sequencing identified four isolates most consistent with *A. calcoaceticus/A. soli/A. seifertei* and four isolates consistent with *A. ursingii/A. septicum*; one isolate was identified as *A. baumannii*. Five of the *Acinetobacter* spp., including the *A. baumannii*, were identified in hospital lobby surfaces. All three of the isolates of *Pseudomonas* spp. came from environmental surfaces near a single hospital; two were most closely identified as *P. putida/fulva* species. One culture, from a mailbox handle, yielded *Stenotrophomonas maltophilia*. The most common organism isolated from the environment was *Brucella* (*Ochrobactrum*) spp. Of the 26 isolates, 15 were identified most closely with *B. intermedia/haematophila* and eight at *B. anthropi*. One isolate of *B. intermedia/haematophila*, from the surface of a vending machine, was found to possess the carbapenemase OXA-24. Outdoor gate and door handles, as well as handrails, were common sites positive for *Brucella* (*Ochrobactrum*) spp. Although seven of the eight hospitals had *Brucella* (*Ochrobactrum*) spp. in their environment, two hospitals accounted for 19 of the 26 cultures. Not one member of the Enterobacterales order was recovered in the environmental cultures.

Cultures from transportation centers near and far medical centers

Among the 40 cultures taken at public transportation centers (i.e. subway stations and bus stops) near hospitals, 38 bacteria were recovered (Table 2). Among eight Acinetobacter isolates, 16S sequencing identified three isolates most consistent with *A. calcoaceticus/A. soli/A. seifertei* and two isolates consistent with *A. ursingii/A. septicum*. No isolates of Pseudomonas were recovered, and two isolates of carbapenem-resistant Stenotrophomonas were isolated. Fifteen of the 38 isolates were *Brucella* (*Ochrobactrum*) spp., including 14 *B. intermedia/haematophila* and one isolate of *B. anthropi*.

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Table 2. Comparison of environmental cultures taken at transportation centers near and distant from medical centers

Isolate	Samples near medical centers	Samples far from medical centers
Number (% carbapenem resistant)		
Achromobacter spp.	2 (0)	2 (0)
Acinetobacter spp.	8 (0)	6 (0)
Brevundimonas spp.	4 (25)	3 (0)
Brucella (*Ochrobactrum*) spp.	15 (20)	5* (20)
Cupriavidus spp.	6 (100)	8 (100)
Pseudomonas spp.	0	2 (50)
Rhizobium/Agrobacterium spp.	1 (0)	2 (0)
Stenotrophomonas spp.	2 (100)	5 (100)

* P = 0.03 compared to samples near medical centers.

A total of 33 isolates were recovered among the 40 cultures taken at transportation centers distant from medical centers. Among six *Acinetobacter* spp., two were identified as *A. baumannii*. There were two *Pseudomonas* spp. isolates and five isolates of carbapenem-resistant *S. maltophilia*. Five isolates were *Brucella* (*Ochrobactrum*) spp., with four identified as *B. pseudogrignonensis*. Compared to the cultures obtained near medical centers, there were significantly fewer isolates of *Brucella* (*Ochrobactrum*) spp. from cultures obtained far from medical centers (5 of 33 vs. 15 of 38, P = 0.03). Once again, no Enterobacterales were isolated in the environmental samples.

Comparison of the survivability of bacteria in desiccated conditions

The survival rates of six bacteria under desiccated conditions were determined. Survival of *E coli* ATCC 25922 and *K pneumoniae* ATCC 700603 was compared to four isolates gathered from the environmental samples: two isolates of *B. intermedia/haematophila* and one isolate each of *P. putida/P. fulva* and *S. maltophilia* (Table 3). With a starting inoculum of ~ 10^6 CFU, survival was considerably poorer for the two members of Enterobacterales (and especially *E. coli*). Over the course of 7 days, there was only a 1–2 log10 CFU decrease in the starting inoculum for the isolates of *Brucella* (*Ochrobactrum*) spp. and *S. maltophilia*.

Discussion

It was reassuring that cephalosporin-resistant Enterobacterales were not recovered from 416 peri-hospital environmental surfaces in our metropolitan region. The routine use of hand hygiene products by hospital employees when leaving the patient care areas may contribute to these findings. Reports of antibiotic-resistant Enterobacterales in the environment have generally emphasized water-related samples, including sink basins, rivers, and wastewater areas (2, 4, 5, 16, 17). Our samples involved 'high-touch' dry surfaces only. As noted in this report, compared to Enterobacterales spp., certain non-fermentative Gram-negative bacteria have a distinct survival advantage in arid environments, likely accounting for our findings. While encouraging that most dry surfaces outside of hospitals are not heavily contaminated with ESBL- or carbapenemase-producing Enterobacterales, testing of standing water and hospital wastewater samples should be done to further evaluate the environmental ecology of these areas.

All of our positive cultures yielded non-fermenting Gram-negative bacilli. Of the 139 positive environmental cultures in this study, 26 (19%) belonged to the genus Acinetobacter, including many to the *A. calcoaceticus – A. baumannii* complex. The *A. calcoaceticus – Acinetobacter baumannii* complex, and in particular *A. baumannii*, has been shown to survive quite well on environmental surfaces within hospitals (7–9). Our cultures were taken during the summer months of July and August in New York City; the high rate of contamination we observed suggests the extra-hospital environment may serve as an important reservoir for hospital-associated Acinetobacter infections, especially during the summer months when there is an increase in Acinetobacter-related infections (18). Similarly, *S. maltophilia* is another important multidrug-resistant hospital pathogen that can survive prolonged periods on desiccated surfaces (12). The finding that 6% of our positive cultures, especially those involving transportation areas, also suggest the extra-hospital environment may be an important reservoir for introduction into the hospital setting. Whole genome sequencing of extra-hospital and clinical isolates of *A. baumannii* and *S. trophomonas* would determine if these isolates are related.

Perhaps our most unanticipated finding was the widespread recovery of *Brucella* (*Ochrobactrum*) spp. The reclassification of *Ochrobactrum* spp. into the Brucella genus in 2022 was followed by considerable controversy (19, 20). Classic *Brucella* spp. (including *B. abortus*, *B. canis*, *B. melitensis*, and *B. suis*) are reportable pathogens that cause animal and human infections (19–21). The reclassified organisms, designated *Brucella* (*Ochrobactrum*) spp., are not reportable, but infections in humans are well described (19–21).

Brucella (*Ochrobactrum*) spp. is well equipped for survival under harsh conditions. Survival in the environment is enhanced by heavy metal tolerance and degradation of xenobiotic compounds including toluene, hexane, and p-xylene (22, 23). In our study, *Brucella* (*Ochrobactrum*) spp. accounted for 39% of the cephalosporin-resistant bacteria isolated in the extra-hospital surfaces and appeared to be territorial. In addition, the proportion of *Brucella* (*Ochrobactrum*) spp. in the transportation centers near the hospitals was significantly greater than that for centers distant from hospitals. Further study will be

Table 3. Decrease in log10 CFU of bacteria over 3 and 7 days at room temperature under desiccant conditions

	E. coli	*K. pneumoniae*	*B. intermedia/haematophila*	*P putida/fulva*	*B. intermedia/haematophila*	*S. maltophilia*
Day 3	6.15	2.06	1.06	1.35	1.00	0.15
Day 7	6.15	3.18	1.76	4.73	1.10	1.79

needed to determine the selective pressures (e.g. environmental cleaning protocols and use of hospital hand hygiene products) responsible for this predominance. Again, whole genome sequencing would be helpful in determining if a single strain is involved in this widespread environmental contamination.

Brucella (*Ochrobactrum*) spp. possesses intrinsic resistance to tetracycline, erythromycin, novobiocin, and because of an AmpC β-lactamase, penicillins, and cephalosporins (22–24). Other resistance genes, including those involving polymyxin and fluoroquinolones, have also been documented (22). Recommended therapies for infections due to *Brucella* (*Ochrobactrum*) spp. include fluoroquinolones, co-trimazole, and imipenem (19, 23, 24). The finding of OXA-24 β-lactamase, a carbapenemase typically found in *Acinetobacter* spp. (25, 26), in one of our isolates suggests that these bacteria can also be reservoirs of clinically important antibiotic resistance genes.

While our study provides reassurance that cephalosporin-resistant Enterobacterales are not widespread on extra-hospital surfaces, we did confirm the presence of an unexpected number of *Acinetobacter* spp. In addition, a surprising number of *Brucella* (*Ochrobactrum*) spp. were recovered, including one harboring an OXA carbapenemase.

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Conflict of interest and funding

None to declare.

Ethics approval

Declared exempt by the SUNY Downstate Institutional Review Board.

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