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MAJOR ARTICLE

Influence of Antibiotics on the Detection of Bacteria by Culture-Based and Culture-Independent Diagnostic Tests in Patients Hospitalized With Community-Acquired Pneumonia

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Background. Specimens collected after antibiotic exposure may reduce culture-based bacterial detections. The impact on culture-independent diagnostic tests is unclear. We assessed the effect of antibiotic exposure on both of these test results among patients hospitalized with community-acquired pneumonia (CAP).

Methods. Culture-based bacterial testing included blood cultures and high-quality sputum or endotracheal tube (ET) aspirates; culture-independent testing included urinary antigen testing (adults) for *Streptococcus pneumoniae* and *Legionella pneumophila* and polymerase chain reaction (PCR) on nasopharyngeal and oropharyngeal (NP/OP) swabs for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. The proportion of bacterial detections was compared between specimens collected before and after either any antibiotic exposure (prehospital and/or inpatient) or only prehospital antibiotics and increasing time after initiation of inpatient antibiotics.

Results. Of 4678 CAP patients, 4383 (94%) received antibiotics: 3712 (85%) only inpatient, 642 (15%) both inpatient and prehospital, and 29 (<1%) only prehospital. There were more bacterial detections in specimens collected before antibiotics for blood cultures (5.2% vs 2.6%; $P < .01$) and sputum/ET cultures (50.0% vs 26.8%; $P < .01$) but not urine antigen (7.0% vs 5.7%; $P = .53$) or NP/OP PCR (6.7% vs 5.4%; $P = .31$). For all diagnostic testing, bacterial detections declined with increasing time between inpatient antibiotic administration and specimen collection.

Conclusions. Bacteria were less frequently detected in culture-based tests collected after antibiotics and in culture-independent tests that had longer intervals between antibiotic exposure and specimen collection. Bacterial yield could improve if specimens were collected promptly, preferably before antibiotics, providing data for improved antibiotic selection.

Keywords. antibiotic stewardship; antibiotic use; bacterial disease; Pneumonia; pneumonia diagnostics.

Community-acquired pneumonia (CAP) is an important infectious cause of morbidity and mortality in the United States [1–3]. Historically, *Streptococcus pneumoniae* has been reported as the leading bacterial cause of CAP. However, since the widespread implementation of pediatric pneumococcal conjugate vaccination in the United States, hospitalizations due to pneumococcal pneumonia have declined in both children and adults [1]. With advances in respiratory virus molecular diagnostics, viruses are more commonly detected than bacteria among patients hospitalized with CAP [4, 5].

The 2007 Infectious Diseases Society of America/American Thoracic Society adult CAP guidelines recommended the routine use of urinary antigen testing for *Legionella pneumophila* serogroup 1 and *S pneumoniae* in addition to blood and sputum cultures in adults hospitalized with severe CAP [2]. The guidelines also recommended starting empiric antibiotic therapy for patients hospitalized with CAP [2]. Empiric antibiotic therapy and a prior Centers for Medicare and Medicaid Services CAP performance standard that mandated the initiation of antibiotic therapy within 6 hours of registration in emergency departments may have de-emphasized diagnostic testing for hospitalized CAP patients [6].

It is generally recommended to start empiric antibiotic therapy for CAP as soon as possible after diagnosis and collect samples for etiology determinations before initiation of therapy. However, data evaluating the impact of prior antibiotic use on bacterial detections in both culture-based and culture-independent diagnostic tests among CAP patients are limited. Some studies indicate that prior antibiotic exposure leads to reduced

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bacterial detections in patients for culture-based diagnostic tests [7, 8]. The low sensitivity of culture-based diagnostic tests compounds this problem [9]. Other studies suggest that the sensitivity of culture-independent diagnostic tests (eg, pneumococcal and *L pneumophila* serogroup 1 urine antigen testing or molecular detection) is not influenced by antibiotic exposure [10, 11].

The Etiology of Pneumonia in the Community (EPIC) study, a large, multicenter, active, population-based surveillance study of hospitalized patients with CAP, systematically captured results of diagnostic testing, antibiotic prescribing, and specimen collection for each patient [4, 5]. We evaluated the influence of antibiotics on both culture-based and culture-independent bacterial diagnostic test results among hospitalized patients with CAP enrolled in the EPIC study.

PATIENTS AND METHODS

The Etiology of Pneumonia in the Community Study

From January 1, 2010 to June 30, 2012, patients hospitalized with clinical and radiographically confirmed CAP were enrolled into the EPIC study [4, 5]. Children <18 years of age were enrolled at 3 hospitals, 1 each in Memphis, Nashville, and Salt Lake City. Adults ≥18 years of age were enrolled at 5 hospitals, 3 in Chicago and 2 in Nashville. Patients were interviewed, medical charts were abstracted, and blood, urine, and respiratory specimens were obtained. Only specimens obtained within 72 hours after admission were included in the analysis. The study protocol was approved by the institutional review board at each institution and the Centers for Disease Control and Prevention.

Culture-Based Diagnostic Tests

Blood for culture was collected from children and adults. Contaminants were previously defined [4, 5]. Endotracheal (ET) aspirates were collected in children and adults only per clinical care. Expectorated sputum specimens were collected for bacterial culture only in adults with productive cough. The pediatric site in Memphis also cultured induced sputum specimens via inhalation of albuterol followed by 7% saline to induce deep cough [12]. Suctioning through the nose or mouth was used in children who were too young to expectorate. For sputum specimens (both expectorated and induced) and ET aspirates, only high-quality specimens were included in the analysis, defined as follows: ≤10 epithelial cells and ≥25 white blood cells/low-power field. Because there were few ET aspirate cultures performed, we combined high-quality sputum and ET aspirate culture results in the analysis. There were only 3 pleural fluid and 4 bronchoalveolar-lavage specimens obtained before antibiotic administration so they were excluded from the analysis.

Culture-Independent Diagnostic Tests

Trained staff obtained nasopharyngeal and oropharyngeal (NP/OP) swabs for real-time polymerase chain reaction (PCR) assays to detect *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Urine specimens from adult CAP patients were tested for *S pneumoniae* and *L pneumophila* serogroup 1 antigens using BinaxNOW. For each specimen obtained, date and time of collection were recorded.

Antibiotic Exposure Assessment

Any patient who self-reported antibiotic use during the 5 days before hospitalization was considered to have prehospital antibiotic exposure. Inpatient antibiotic exposure, including date and time of administration of the first inpatient antibiotic, was abstracted from the medical charts. For analyses involving *C pneumoniae* and *M pneumoniae*, antibiotics were grouped into classes with (macrolide or fluoroquinolone) and without (β-lactams, clindamycin, other) activity against these atypical bacteria. Specimens with no date or time documented for antibiotic administration or specimen collection were excluded.

Analytic Approach

First, we determined the number of patients who received antibiotics in inpatient, prehospital, or both settings, and we compared the proportion of bacterial detections among specimens collected before and after either inpatient, prehospital, or any (inpatient and/or prehospital) antibiotic exposure for each diagnostic test. Second, to assess the influence of prehospital antibiotic exposure, we excluded specimens that were collected after inpatient antibiotic exposure and compared the proportion of detections among specimens collected with and without prehospital antibiotic exposure. Third, to examine the effect of time after first antibiotic exposure on detections, we evaluated diagnostic yield at different time points. We excluded specimens with prehospital antibiotic exposure and divided exposure time into quartiles and compared the proportion of bacterial detections in each quartile to the proportion in specimens with no antibiotic exposure. The χ^2 or Fisher's exact tests were used for comparisons as appropriate. The Cochran-Armitage trend test was used to compare the proportion of bacterial detections over time. All comparisons were 2-sided, and a P value of <.05 was considered significant. Data were analyzed using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

There were 4676 hospitalized patients with radiographically confirmed CAP (2320 adults and 2356 children) enrolled. Table 1 shows patients receiving antibiotics by inpatient and/or prehospital setting.

There were 275 (6.5%) of 4246 blood cultures, 17 (3.8%) of 445 sputum/ET cultures, 26 (1.3%) of 1941 urine antigen tests, and 320 (7.0%) of 4550 NP/OP samples excluded because of unknown date and time of antibiotic administration. The number and proportion of specimens collected before prehospital or inpatient antibiotics included the following: 2679 (67.5%) of 3971 blood cultures, 36 (8.4%) of 428 sputum/ET cultures, 158 (8.3%) of 1915 urine antigen tests, and 405 (9.6 %) of 4230 NP/OP samples.

Table 1. Proportion of Adults and Children Hospitalized With Community-Acquired Pneumonia Who Received Antibiotics

Antibiotics receipt by setting[b]	Adult, n (%)	Child, n (%)	Total, N (%)
n (row %)	2320 (49.6)	2356 (50.4)	4676 (100)
n (column %)			
Received inpatient antibiotics only	2043 (88.1)	1667 (70.7)	3710 (79.3)
Received prehospital[a] antibiotics only	2 (0.1)	27 (1.1)	29 (0.6)
Received both inpatient and prehospital[a] antibiotics	244 (10.5)	398 (16.9)	642 (13.7)
No antibiotics received	31 (1.3)	264 (11.2)	295 (6.3)

[a]Prehospital antibiotic exposure was defined as receiving an antibiotic during the 5 days before admission.

[b]For 61 patients, it was unknown whether they received inpatient and/or prehospital antibiotic.

Culture-Dependent Tests

There were 139 (5.2%) bacterial detections in 2679 blood cultures collected before any antibiotics (prehospital and/or inpatient) compared with 33 (2.6%) bacterial detections in 1292 blood cultures collected after any antibiotics ($P < .01$) (Table 2).

Detection of *S pneumoniae* was lower among blood cultures collected after any antibiotics (Table 2) compared with those collected before antibiotics. For children, 37 of 1164 blood cultures collected before antibiotics were positive compared with 14 of 725 blood cultures collected after antibiotics (3.2% vs 1.9%;

Table 2. Proportion of Bacterial Detections Based on Specimens Collected Before and After Inpatient and/or Prehospital Antibiotics for Each Diagnostic Test

	Specimens Obtained Before Antibiotics					
	Yes (%)			No (%)		
Diagnostic Tests	Adult	Child	Total	Adult	Child	Total
Blood cultures, n	1515	1164	2679	567	725	1292
Total bacterial detections	102 (6.7)[c]	37 (3.2)	139 (5.2)[d]	19 (3.4)	14 (1.9)	33 (2.6)
Streptococcus pneumoniae	37 (2.4)	18 (1.5)[c]	55 (2.1)[c]	7 (1.2)	2 (0.3)	9 (0.7)
Streptococcus pyogenes	4 (0.3)	3 (0.3)	7 (0.3)	2 (0.4)	1 (0.1)	3 (0.2)
Streptococcus aureus	18 (1.2)	3 (0.3)	21 (0.8)	3 (0.5)	3 (0.4)	6 (0.5)
Haemophilus influenzae	7 (0.5)	1 (0.1)	8 (0.3)	2 (0.4)	2 (0.3)	4 (0.3)
Other pathogens[a]	36 (2.4)[c]	13 (1.1)	49 (1.8)[c]	5 (0.9)	7 (1.0)	12 (0.9)
High-quality sputum or ET cultures, n	13	23	36	260	132	392
Total bacterial detections	2 (15.4)	16 (69.6)	18 (50.0)[c]	29 (11.2)	76 (57.6)	105 (26.8)
S pneumoniae	2 (15.4)	4 (17.4)	6 (16.7)	11 (4.2)	18 (13.6)	29 (7.4)
S pyogenes	0 (0.0)	1 (4.3)	1 (2.8)	3 (1.2)	3 (2.3)	6 (1.5)
S aureus	0 (0.0)	6 (26.1)	6 (16.7)	11 (4.2)	29 (22.0)	40 (10.2)
H influenzae	0 (0.0)	6 (26.1)	6 (16.7)[c]	0 (0.0)	23 (17.4)	23 (5.9)
Other pathogens[b]	0 (0.0)	10 (43.5)[c]	10 (27.8)[d]	4 (1.5)	32 (24.2)	36 (9.2)
Urine antigen test, n (only performed in adults)	158		158	1757		1757
Total detections	11 (7.0)	NA	11 (7.0)	101 (5.7)	NA	101 (5.7)
Pneumococcal antigen detection	7 (4.4)	NA	7 (4.4)	74 (4.2)	NA	74 (4.2)
Legionella antigen detection	4 (2.5)	NA	4 (2.5)	26 (1.5)	NA	26 (1.5)
Combined pneumococcal and *Legionella* antigen detections	0 (0.0)	NA	0 (0.0)	1 (0.1)	NA	1 (0.1)
NP/OP swab PCR assay, n	126	279	405	2118	1707	3825
Total *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* detections	7 (5.6)[c]	20 (7.2)	27 (6.7)	45 (2.1)	163 (9.5)	208 (5.4)

Abbreviations: ET, endotracheal tube; NA, nonapplicable; NP/OP, nasopharyngeal and oropharyngeal; PCR, polymearase chain reaction.

Columns may not add to 100% because some specimens may have had more than 1 pathogen detected. The following bacteria were considered contaminants and were excluded from the analyses: *Aerococcus*, *Alcaligenes*, *Bacillus*, *Citrobacter*, coagulase-negative *Staphylococcus*, *Corynebacterium*, *Enterococcus*, *Micrococcus*, *Neisseria subflava*, *Propionibacterium*, *Stomatococcus*, *Streptococcus bovis*, and *Veillonella*.

[a]Other pathogens (specimens before antibiotics: yes/no): *Escherichia coli* (13/0); viridans streptococci species (9/4); *Klebsiella* (6/0); streptococcal groups B, C, or G (7/3); *Fusobacterium* (3/1); *Pseudomonas* (2/1); *Moraxella* (1/1); *Acinobacter* (2/0); *Enterobacter* (1/0); *Pasteurella* (1/0); *Proteus* (1/0); or codetections (3/2).

[b]Other pathogens (specimens before antibiotics: yes/no): *Moraxella* (10/30), *Pseudomonas* (0/4), *E coli* (0/1), *Enterobacter* (0/1).

[c]*P* value <.05 compared with specimens obtained after antibiotics.

[d]*P* value <.001 compared with specimens obtained after antibiotics.

P = .10). For adults, 102 of 1515 blood cultures collected before antibiotics were positive compared with 19 of 567 blood cultures collected after antibiotics (6.7% vs 3.4%; P < .01). Analyzing the effect of prehospital antibiotics, there were 147 positive blood cultures from 3073 specimens collected from patients without prehospital antibiotic exposure compared with 16 positive blood cultures from 600 specimens from patients who reported taking prehospital antibiotics (4.8% vs 2.7%; P = .02).

With increasing time between inpatient antibiotic administration and specimen collection, the proportion of blood cultures with a bacterial detection decreased with increased time; the proportion positive was 5.2% for specimens collected before inpatient antibiotic administration compared with 1.7% for specimens collected >15 hours after initial inpatient antibiotic exposure (P for trend <.01) (Table 3).

There were 18 (50.0%) bacterial detections in 36 high-quality sputum/ET specimens collected before antibiotics compared with 105 (26.8%) bacterial detections in 392 sputum/ET specimens collected after antibiotics (prehospital and/or inpatient) (P < .01) (Table 2). For children at the Memphis site, 16 of 23 sputum/ET specimens collected before antibiotics yielded pathogens compared with 76 of 132 sputum/ET specimens collected after antibiotics (69.6% vs 57.6%; P = .28). For adults, 2 of 13 sputum/ET specimens collected before antibiotics yielded pathogens compared with 29 of 260 sputum/ET specimens collected after antibiotics (15.4% vs 4.2%; P = .24).

Analyzing the effect of prehospital antibiotics, 47 of 90 sputum/ET cultures from specimens collected without prehospital antibiotic exposure yielded pathogens compared with 8 of 49 sputum/ET cultures from patients who reported taking prehospital antibiotics (52.2% vs 16.3%; P < .01).

With increasing time between inpatient antibiotic administration and specimen collection, the proportion of sputum specimens with a bacterial detection decreased from 50.0% for specimens collected before antibiotic exposure compared with 12.2% for specimens collected >20 hours after initial inpatient antibiotic exposure (P for trend <.01) (Table 3).

Table 3. Proportion of Bacterial Detections Comparing Specimens Collected Before and After Inpatient Antibiotic Administration According to Time Elapsed Between Antibiotic Administration and Specimen Collection Stratified Into Quartiles[a]

Blood cultures (n = 3369)	n	Bacterial Detections (%)	P Value for Trend[b]
Before antibiotics	2679	139 (5.2)	<.01
>0–1 hours after antibiotics	163	8 (4.9)	
>1–4 hours after antibiotics	176	5 (2.8)	
>4–15 hours after antibiotics	176	1 (0.6)	
>15 hours after antibiotics	175	3 (1.7)	
ET/Sputum Cultures (n = 378)			
Before antibiotics	36	18 (50.0)	<.01
>0–5 hours after antibiotics	98	46 (46.9)	
>5–10 hours after antibiotics	76	23 (30.3)	
>10–20 hours after antibiotics	86	17 (19.8)	
>20 hours after antibiotics	82	10 (12.2)	
Urine Pneumococcal or *Legionella* test (n = 1693)			
Before antibiotics	157	11 (7.0)	.01
>0–6 hours after antibiotics	374	33 (8.8)	
>6–15 hours after antibiotics	395	22 (5.6)	
>15–23 hours after antibiotics	363	19 (5.2)	
>23 hours after antibiotics	404	14 (3.5)	
NP/OP PCR for *Mycoplasma pneumoniae* or *Chlamydia pneumoniae* (n = 3557)			
Before antibiotics	401	27 (6.7)	.01
>0–5 hours after antibiotics	752	39 (5.2)	
>5–14 hours after antibiotics	845	29 (3.4)	
>14–21 hours after antibiotics	739	27 (3.7)	
>21 hours after antibiotics	820	28 (3.4)	

Abbreviations: ET, endotracheal tube; NP/OP, nasopharyngeal and oropharyngeal; PCR, polymearase chain reaction.

[a]Patients with prehospital antibiotic exposure, and/or unknown timing of inpatient antibiotic exposure, were excluded from analysis.

[b]Cochran-Armitage trend test.

Culture-Independent Tests

Of 158 urine specimens collected before antibiotics, there were 11 (7.0%) urinary pneumococcal or *L pneumophila* serogroup 1 detections, not significantly different from specimens collected after antibiotics (inpatient and/or prehospital) (101 of 1757 [5.7%]; P = .53) (Table 2). We found that urinary antigen detection rates were similar when comparing specimens with and without prehospital antibiotic exposure (6.8% vs 6.0%; P = .74). With increasing time between inpatient antibiotic administration and specimen collection, the proportion of pneumococcal or *L pneumophila* serogroup 1 antigen detections decreased from 7.0% for specimens collected before antibiotic exposure to 3.5% for specimens collected >23 hours after initial inpatient antibiotic exposure (P for trend <.01) (Table 3).

Mycoplasma pneumoniae and *C pneumoniae* were detected by PCR from 27 (6.7%) of 405 NP/OP specimens collected before antibiotics compared with 208 (5.4%) detected in 3825 NP/OP specimens collected after antibiotics (prehospital and/or inpatient) (P = .31) (Table 2). Among adults, *M pneumoniae* or *C pneumoniae* was detected in 7 of 126 NP/OP specimens collected before antibiotics and 45 of 2118 NP/OP specimens collected after antibiotics (5.6% vs 2.1%; P < .05). For children, there were 20 bacterial detections in 279 NP/OP specimens collected before antibiotics and 163 bacterial detections in 1707 NP/OP specimens collected after antibiotics (7.2% vs 9.5%; P = .20). The proportion of *M pneumoniae* or *C pneumoniae* detection was significantly higher in children receiving either a fluoroquinolone or macrolide antibiotic class and was lower for children receiving other antibiotic classes (Figure 1A). Subgrouping children further, we found a similar yield before and after antibiotics for young children under 5 years (4.5% vs 3.9%; P = .65); however, for children 5 to 17 years, we found 11 detections in 81 specimens collected before antibiotics compared with 96 detections in 338 specimens collected after antibiotics (13.6% vs 21.8%; P = .08). This finding became statistically significant

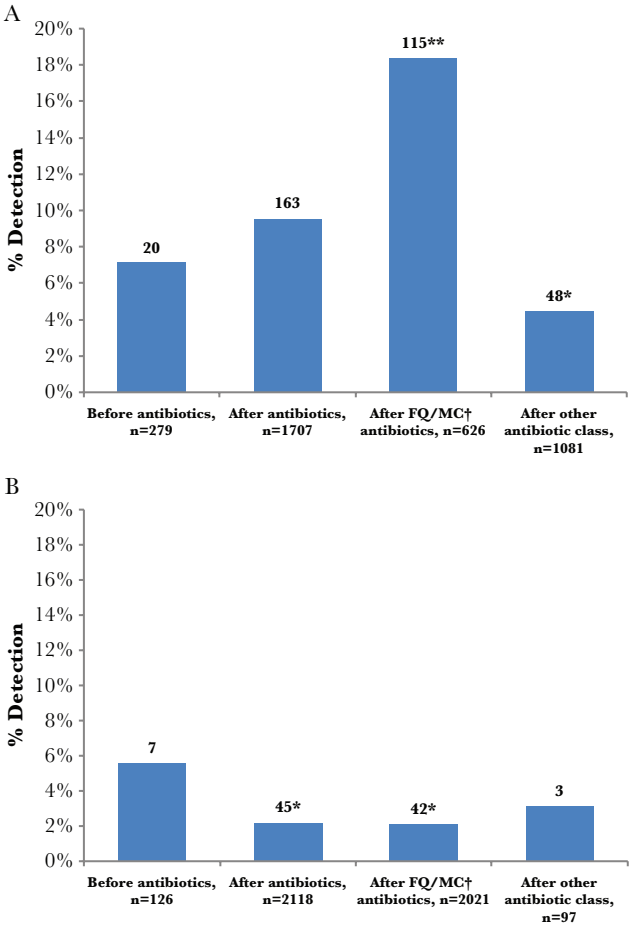

Figure 1. (A) Proportion of *Chlamydia pneumoniae* or *Mycoplasma pneumoniae* detections among nasopharyngeal and oropharyngeal (NP/OP) specimens collected before and after inpatient and/or prehospital antibiotics overall and by antibiotic class in children. Numbers above bars represent the number of bacterial detections. (B) Proportion of *C pneumoniae* and *M pneumoniae* detections among NP/OP specimens collected before and after inpatient and/or prehospital antibiotics overall and by antibiotic class in adults. Numbers above bars represent the number of bacterial detections. ‡NP/OP swabs for real-time polymerase chain reaction assays to detect *C pneumoniae* and *M pneumoniae*. †FQ/MC, fluoroquinolone or macrolide. **P* value <.05 compared with specimens obtained before antibiotics. ***P* value <.001 compared with specimens obtained before antibiotics.

when we compared the yield before antibiotics to yield after exposure to a fluoroquinolone or macrolide antibiotic (13.6% vs 28.4%; $P < .01$) for children aged 5 to 17 years. For adults, the proportion of *M pneumoniae* or *C pneumoniae* detections was lower in adults who received either a fluoroquinolone or macrolide antibiotic before NP/OP specimen collection compared with specimens collected after antibiotics (Figure 1B).

Analyzing the effect of prehospital antibiotics, we found that PCR detected *M pneumoniae* or *C pneumoniae* in 51 of 913 NP/OP specimens collected from patients without prehospital antibiotic exposure compared with 81 of 647 specimens from patients who reported taking prehospital antibiotics (5.6% vs 12.5%; $P < .01$). With increasing time between inpatient antibiotic administration and specimen collection, the proportion of *M pneumoniae* and *C pneumoniae* detections decreased from 6.7% for specimens collected before antibiotic exposure compared with 3.4% for specimens collected >21 hours after initial antibiotic exposure (*P* for trend <.01) (Table 3).

DISCUSSION

Our analysis showed that bacteria were less frequently detected in culture-based tests collected after antibiotic exposure and for culture-independent tests that had longer intervals between antibiotic exposure and specimen collection. Antibiotic exposure was associated with reduced bacterial yield from blood cultures, sputum, and tracheal aspirates by approximately 50%. The incremental increase in time after inpatient antibiotic exposure was also associated with decreased bacterial yield, emphasizing the need to obtain cultures as close to the time of antibiotic administration as possible. Antibiotic administration did not appear to be associated with yield from urinary antigen detection assays for *S pneumoniae* or *L pneumophila* serogroup 1. Among adults but not children, antibiotic exposure, particularly exposure to fluoroquinolones or macrolides, was associated with decreased yield from PCR assays for *M pneumoniae* and *C pneumoniae*.

In our study, most blood cultures were collected before antibiotics, and we found a lower proportion of bacterial detections in specimens collected after antibiotics. Historically, studies have shown that 6%–16% of hospitalized patients with CAP had documented bacteremia [13–16]. In the EPIC study, 2.5% of children and 5.7% of adults had documented bacteremia [4, 5]; the decreased proportion of bacterial detections may be a result of widespread implementation of pediatric pneumococcal conjugate vaccination. In our analysis, blood cultures collected before antibiotics were positive in 6.7% of adults and 3.2% of children. This dropped substantially after prehospital and/or inpatient antibiotics, similar to a surveillance study of 35 639 patient blood cultures from 29 883 hospitalized patients with pneumonia in Thailand that reported a 4.1% drop in pathogen detections [7]. We found a decreased proportion of bacterial detections as the time between antibiotic exposure and specimen collection increased, and this became significant >4 hours between antibiotic exposure and specimen collection.

Studies have shown that the diagnostic yield for sputum cultures varies from 10% to 86% depending upon specimen quality and previous antibiotic use [17]. A small study of 85 hospitalized adult patients in the United States with bacteremic pneumococcal pneumonia found that the frequency of pneumococcal growth in sputum specimens decreased with increasing time from antibiotic initiation [18]. A study among elderly hospitalized patients with CAP also showed a decrease in *S pneumoniae* detection after antibiotic exposure when using culture-based diagnostic tests [19]. In our study, the proportion of *S pneumoniae* was lower in high-quality sputum/ET specimens collected after antibiotics, although this was not statistically significant. To maximize diagnostic yield for culture-based

diagnostic testing, specimens should be collected before or as soon as possible after antibiotic administration.

For the culture-independent urine antigen tests for *S pneumoniae* and *L pneumophila* serogroup 1 in adults, there were fewer detections in specimens collected after antibiotics compared with before, although this was not statistically significant. Urinary antigen tests for pneumococcus were responsible for the majority (67%) of pneumococcal detections in the EPIC study [4]. The urine antigen diagnostic tests offer both high sensitivity and specificity for identifying pneumococcus or *L pneumophila* serogroup 1 in adult patients with CAP [17]. Previous studies have reported continued antigen detection despite antibiotic exposure [11, 19]. Although we found a lower proportion of urine antigen detections after antibiotic exposure, this was not statistically significant. Other studies have reported fewer urinary pneumococcal antigen detections after prehospital antibiotic exposure [8, 20]. When we examined urine antigen detections based on self-reported prehospital antibiotic exposure within 5 days before admission, we found no significant difference in detections before or after antibiotic exposure. *Streptococcus pneumoniae* remains an important cause of bacterial CAP [4, 5]; thus, early and increased use of rapid pneumococcal urine antigen tests for adults with CAP could improve antibiotic selection and allow for subsequent antibiotic de-escalation [21, 22]. However, a recent national survey among practicing US infectious disease clinicians suggested that the urine pneumococcal antigen test is only used by 65% of providers [23]. Urinary antigen testing for *S pneumoniae* or *L pneumophila* serogroup 1 in an adults hospitalized with CAP should be pursued in accordance with current clinical guidelines [2], although we observed false-positive results in some patients who received 23-valent pneumococcal polysaccharide vaccine before urine collection in the EPIC study [24].

Atypical bacterial pathogens were the most common bacteria detected among pediatric patients hospitalized with CAP in the EPIC study [5], and these were detected in 8% of children and 2% of adults (4, 5). Although multiplex PCR is often used for respiratory virus detection, atypical bacteria have not routinely been included in these panels for clinical use [17]. However, a multiplex PCR for detection of *M pneumoniae* and *C pneumoniae* was approved by the US Food and Drug Administration in 2013 [25], which could help inform antibiotic use and choice. In our study, only 10.6% of these research NP/OP specimens were collected before antibiotic exposure. Although more atypical bacteria were detected by PCR in specimens exposed to prehospital antibiotics compared with specimens not exposed, when we excluded specimens with prehospital antibiotic exposure, we found the proportion of *M pneumoniae* and *C pneumoniae* detections by PCR in NP/OP specimens significantly decreased with increasing time after antibiotic exposure compared with specimens collected before antibiotics. When examined by age, adults also had significantly fewer PCR detections when exposed to antibiotics. Although children more frequently had atypical bacterial detection by PCR after antibiotic exposure, higher detection was primarily in older children aged 5–17 years who received a fluoroquinolone or a macrolide. Potential explanations include clinicians choosing a macrolide or fluoroquinolone in children with a heightened risk of atypical bacterial infection, or that fluoroquinolones or macrolides may result in bacterial killing that releases the molecular material in the specimen increasing the likelihood of a PCR-based detection.

Our study was subject to several limitations. First, the EPIC study was not specifically designed to answer the question of whether pretest antibiotic exposure effects bacterial detection, and thus serial samples on the same patient collected before and after antibiotic exposure were not obtained. Second, the analysis is subject to uncontrolled confounding for patient characteristics associated with either earlier or later timing of specimen collection and/or antibiotic administration. In addition, although specimens, particularly blood, were collected as soon as possible from patients per clinical care, NP/OP, sputum, and urine specimens were often collected at the time of enrollment and after informed consent per the study protocol. Third, certain specimen types included in this study are not routinely collected in clinical practice such as expectorated sputum, ET aspirates, urine, and NP/OP swab specimens, which may limit the generalizability of our findings. Fourth, a recall bias might exist in patients self-reporting prehospital antibiotic use.

CONCLUSIONS

In conclusion, the frequency of bacterial detections decreased with increasing time between inpatient antibiotic exposure and specimen collection for culture-based diagnostic tests, as well as for *M pneumoniae* and *C pneumoniae* PCR detection in adult NP/OP specimens. Urinary antigen testing for *L pneumophila* and *S pneumoniae* was less effected by antibiotic exposure, and these tests should be used in accordance with clinical guidelines. Our results suggest that bacteria could be detected more commonly if specimens were collected before or early after administering antibiotic therapy, leading to improved pathogen detection and targeted antibiotic therapy. Newer rapid and accurate bacterial diagnostics, especially culture-independent tests, are urgently needed for hospitalized CAP to improve pathogen detection and better inform and facilitate antibiotic de-escalation [26].

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and interpretation, critically reviewed the manuscript, and approved the final manuscript as submitted. S. J. obtained funding to conduct the study, supervised the study, helped design the study, helped analyze and interpret the data, critically reviewed and revised the manuscript, and approved the final manuscript as submitted. L. A. H. contributed to study design, supervised A. M. H. in the analysis and interpretation of the data, critically reviewed the manuscript as a subject matter expert, and approved the final manuscript as submitted. S. R. A., K. A., W. H. S., D. J. W., E. J. A., C. G. G., J. A. M., A. T. P., R. G. W., and K. M. E. obtained funding to conduct the study, enrolled patients and collected data at the study sites, contributed to study design, helped interpret the data, critically reviewed the manuscript, and approved the final manuscript as submitted.

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