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Variability of Adenosine Triphosphate–Based Bioluminescence Assay Readings among Drug-Resistant Pathogens

To the Editor—Environmental contamination of clinically relevant drug-resistant organisms (eg, methicillin-resistant *Staphylococcus aureus* [MRSA], *Clostridium difficile*, vancomycin-resistant enterococci [VRE], and extended-spectrum β-lactamase [ESBL]–producing and *Klebsiella pneumoniae* carbapenemase [KPC]–producing gram-negative bacteria) frequently occurs and may contribute to their transmission in the healthcare setting.[1-3] The Centers for Disease Control and Prevention has recommended that hospitals ensure compliance by housekeeping staff with cleaning and disinfection procedures.[4] Monitoring systems have been developed to determine the effectiveness of hospital cleaning procedures.[5] One such system utilizes the detection of adenosine triphosphate (ATP) on surfaces. ATP is a compound used in the metabolic processes of cells and, therefore, is present in all organic material. The detection of ATP on environmental surfaces in hospitals has been used to assess the adequacy of routine cleaning procedures, with a proposed cutoff value of less than 250 relative light units (RLUs) for a thoroughly cleaned surface.[6,7] This study aimed to determine whether differences in detectability based on ATP readings of epidemiologically significant organisms, including drug-resistant pathogens, can be found.

An ATP bioluminescence assay, the AccuPoint HC system (Neogen), was used to assess differences among 9 known bacterial strains obtained from the American Type Culture Collection (ATCC)—namely, MRSA (ATCC 43300), VRE (ATCC 51299), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 38657), KPC-producing *K. pneumoniae* (ATCC BAA-1705), ESBL-producing *K. pneumoniae* (ATCC 700603), *C. difficile* vegetative form (ATCC 43255), and *C. difficile* spores (ATCC 43255). These were prepared to an approximate concentration of 1.5×10^6/10 μL. For each bacterial strain, four 1 : 10 serial dilutions were made from the base suspension. ATP readings were then taken from the base suspension and from each dilution by inoculating 10 μL onto the ATP swab. Ten replicates were done for each concentration. Ten-microliter samples from each dilution of organisms were grown to determine the viability of the organisms and the accuracy of the dilution process.

Our results (Table 1) showed that the ATP readings were proportional to the concentration values for MRSA, VRE, and *P. aeruginosa*. With concentrations of 1.5×10^6/10 μL, ATP readings were highest, and these declined proportionately to the 1.5×10^2/10 μL values. This is a validation of the bacterial concentration. KPC-producing *K. pneumoniae* and *C. difficile* spores were undetected at any concentration. For *E. coli*, *K. pneumoniae*, and ESBL-producing *K. pneumoniae*, values were detectable only at the highest concentration (1.5×10^6/10 μL); for vegetative *C. difficile*, values were detectable only at the highest 2 concentrations (1.5×10^6/10 μL and 1.5×10^5/10 μL).

It is clear from these data that for KPC-producing *K. pneumoniae* and *C. difficile* spores, no concentration examined yielded measurable ATP values. For the remaining 7 bacteria, a simple analysis of variance was conducted with the highest concentration to verify the clear differences among the different bacteria. The differences were significant among the pathogens with measurable ATP readings ($F = 41.3$, $P \leq .001$).

We demonstrated differences in detectability and ATP readings of the organisms studied. A value of 250 RLUs has been proposed by previous studies as thoroughly "clean."[6,7] However, this standard may need to be assessed further for eval-

TABLE 1. Differences in Adenosine Triphosphate Bioluminescence Readings among Hospital-Acquired Pathogens, Including Drug-Resistant Organisms, at Different Concentrations

Organism	Concentration				
	$1.5 \times 10^6/10\ \mu L$	$1.5 \times 10^5/10\ \mu L$	$1.5 \times 10^4/10\ \mu L$	$1.5 \times 10^3/10\ \mu L$	$1.5 \times 10^2/10\ \mu L$
MRSA	1,985 ± 668	219.5 ± 163.4	46.8 ± 39.0	3.2 ± 10.1	0
VRE	648.9 ± 230.8	41.5 ± 17.6	2.2 ± 3.6	0.6 ± 1.9	0.6 ± 1.9
Pseudomonas aeruginosa	820.3 ± 616.9	63.7 ± 37.0	4.1 ± 8.7	0	0
Escherichia coli	33.6 ± 16.7	0	0	0	0
Klebsiella pneumoniae	47.7 ± 63.9	0	0	0	0
KPC-producing *K. pneumoniae*	0	0	0	0	0
ESBL-producing *K. pneumoniae*	102.1 ± 57.9	0	0	0	0
Clostridium difficile	11.0 ± 21.1	2.2 ± 3.3	0	0	0
C. difficile spores	0	0	0	0	0

NOTE. Data are mean relative light units ± standard deviation. ESBL, extended-spectrum β-lactamase; KPC, *Klebsiella pneumonia* carbapenemase; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococcus.

uation of patient rooms previously occupied by patients with specific resistant pathogens. Our results were similar to those of a study by Turner et al[8] that demonstrated differences in RLU readings between *S. aureus* and *E. coli* as well as overall weak detection of pure organisms. At bacterial counts of approximately $1.5 \times 10^6/10\ \mu L$, MRSA yielded the highest RLU reading, followed by *P. aeruginosa* and VRE. All other strains tested yielded readings that would be classified as clean on the basis of a cutoff value of 250 RLUs.

Although it is almost impossible to encounter contamination of a hospital environment by pure culture, it is important to remember this limitation of the test. This test should always be utilized in conjunction with good infection control practices, including adequate housekeeping education and adherence to cleaning practices. Potentially, a room previously occupied by a patient with *C. difficile* may exhibit low RLU readings and therefore be deemed as "clean." However, if nonsporocidal agents were inappropriately used, viable spores unmeasured by the assay can be left behind and result in transmission.

Our study has several limitations. First, we studied only specific strains of organisms. It is possible that organisms of the same species may also have variability in ATP bioluminescence among different strains. Second, we examined pure bacterial isolates in the laboratory, and readings in the actual environment may vary.

In summary, we found that there are significant differences in ATP readings among pathogens. Although this technology has been well established in the food industry, its use in hospitals has been instituted more recently. These findings add to our understanding of these commercially available products as we see their increasing use in the healthcare setting.

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Increasing Influenza Vaccination Rates among Hospital Employees without a Mandatory Policy

To the Editor—Influenza vaccination is the best way to protect against influenza infection.[1] For healthcare workers, the Centers for Disease Control and Prevention (CDC), the Advisory Committee on Immunization Practices (ACIP), and the Healthcare Infection Control Practices Advisory Committee (HICPAC) all recommend that US healthcare workers get vaccinated annually. During the 2010–2011 influenza season, the influenza vaccination rate among healthcare workers was estimated at 63.5%. However, the rate increased to over 98% when there was a requirement for vaccination by their employers.[1] Because of this discrepancy, the Society for Healthcare Epidemiology of America (SHEA) endorsed a policy in which annual influenza vaccination should be "a condition of both initial and continued healthcare personnel employment and/or professional privileges."[2] Many hospitals have been reluctant to institute such a policy on the basis of both the fear of litigation and the compromising of employees' civil liberties and autonomy.[3]

Virginia Hospital Center (VHC) is a 334-bed teaching hospital in Arlington, Virginia, located in the Washington, DC, metropolitan area. During the 2011–2012 influenza season, there were an estimated 2,723 hospital employees, of which about 20% had nonclinical roles. Although the hospital administration assured their full support of any influenza vaccination program, they were reluctant to implement a mandatory vaccination policy. We report our experience on trying to improve vaccination rates without instituting an official requirement.

In previous years, VHC has provided free influenza vaccine, available at different times and locations via mobile carts and centralized dispensing areas. The hospital has also publicized the availability and importance of vaccination at monthly leadership meetings and in the form of posters, e-mail alerts, hospital newsletters, and overhead announcements. Additionally, the use of declination letters has been added to the influenza plan in an effort to increase vaccination rates. Despite these efforts, during the 2010–2011 influenza season, the vaccination rate was 61%, a rate much lower than those achieved in hospitals with mandatory vaccination programs.[4,5,6]

In the months prior to the 2011–2012 influenza season, an influenza task force was created, consisting of members of the Infection Prevention Committee, Employee Health, Medical Staff Office, Pharmacy, Public Affairs, and the hospital administration. A comprehensive vaccination policy was instituted that included methods from previous years as well as 3 additional components: stickers on badges identifying whether individuals were vaccinated or not; use of surgical masks by unvaccinated employees when within 6 feet of a patient; and weekly e-mails to department supervisors updating them on their employees' status. It was required that employees either receive the vaccine or sign a declination letter. Documentation of vaccination outside VHC was acceptable for those vaccinated elsewhere. Additionally, department supervisors had the responsibility to ensure all of their employees took some sort of action. Vaccination started October 1, 2011, and employees were encouraged to take action by December 1, 2011.

During the 2011–2012 influenza season, 2,306 employees received the vaccine or showed proof of vaccination elsewhere, and 141 signed a declination letter, resulting in a 90% compliance rate with hospital policy. Overall, the vaccination rate was 85% (Table 1). The most common reason for declination was "Personal/Do not want." There was no disciplinary action taken against those who were noncompliant with hospital policy. Vaccination rates were 86% in clinical employees compared to 74% in nonclinical employees. One vaccinated employee developed a cough 1 hour after vaccination and was seen in the emergency department. She had no lip or tongue swelling on physical examination and had no wheezing upon auscultation. She was given a short course of steroids for a possible allergic reaction. Nonhospital employees such as students and hospital contractors were provided free vaccine, and vaccination rates were well over 90% for this population.

We saw a dramatic increase in influenza vaccination rates during the 2011–2012 season, despite not implementing a mandatory vaccination policy. Although we did require some action to be taken, there was no consequence if an employee chose not to do anything or if a supervisor did not enforce the policy. However, because of the aggressive marketing of the vaccine and the requirement to wear a mask if unvaccinated, many

TABLE 1. Influenza Vaccination among Employees over a 5-Year Period

Year	Employees vaccinated	Total no. of employees	Vaccination rate, %
2007	932	2,053	45
2008	1,034	2,358	44
2009	1,237	2,165	57
2010	1,363	2,239	61
2011	2,306	2,723	85

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