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Scientific Research

A New Epidemiological Tool for *Staphylococcus aureus* Surveillance

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Abstract

Epidemiological surveillance for microbes is currently based on either agar culture followed by identification, or genetic amplification. Both techniques are highly skilled-labor intensive, costly, and must be done in central laboratories. The Defined Substrate Utilization® (DSU®) format provides an epidemiological series of specific screening formulations that obviate these limitations. All reagents are present in optimized stable powder form in a test tube—add water, inoculate, and incubate. A specific color change provides a sensitive and specific detection of the target microbe. Two DSU® methods for *Staphylococcus aureus* (*S. aureus*) are presented: aureusAlert® for all *S. aureus* and EPI-M® for methicillin-resistant *S. aureus* (MRSA). Both aureusAlert® and EPI-M® had a detection level of 20 colony forming units (CFU) in 18 hours. aureusAlert® and conventional methods agreed 93.6% and EPI-M® and conventional methods agreed 94.1%. DSU® and conventional methods showed the same specificity.

Keywords

MRSA, *Staphylococcus aureus*, Methicillin-Resistant, Epidemiology

1. Introduction

There are two basic protocols utilized for the epidemiological screening of microbes. The first is the conventional agar-based culture technique. Here a specimen is plated on one or more selective agars and incubated 24 to 48 hours. A trained medical technologist examines the colonies and those compatible with the target microbe are subjected to specific biochemical and/or immunochemical testing, and antibiotic susceptibility procedures. These may require from 24 to 72 hours incubation [1]. In an attempt to facilitate the culture protocol, chromo-

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genic substrate media have been introduced for some, but not all, bacteria of epidemiological interest. Chromogenic substrate agars have a short shelf life and must be stored cold and often in the dark, and the colors differentiating the positive and negative colonies may not always be distinct, necessitating confirmatory testing [2]. They are often held 48 hours before calling a negative, although 48 hours can result in the generation of false positives [3] [4].

The second technique utilizes genetic amplification (GA) to detect the target microbe. A number of companies make different types of GA. However, in common to them all are: each requires an expensive instrument, utilizes reagents which are expensive, requires highly skilled labor in a centralized laboratory, and does not recover living bacteria [5]. In addition, each is subject to false positives and false negatives because of unexpected mutations [6] [7].

A new and novel, one-step, direct epidemiology screening method based on classical phenotypic parameters was developed as an alternative to conventional agar-based methods. For *S. aureus*, the key enzyme is coagulase and specificity is achieved equal to that of conventional identification schemes [8]. The method of Defined Substrate Utilization® (DSU®) optimizes specific, selective, and differential biochemicals in powder form. Hence, it overcomes the observation by Selepak and Witebsky that the lot-to-lot variation of commercial plasma was too variable to be used directly from patient specimens [9].

Therefore, aureusAlert® is the first direct, one-step, specimen screening tool for viable *S. aureus*. The powder format allows optimization of ingredients much more precisely than an agar gel. Moreover, the overall sensitivity of the DSU® is enhanced since liquid culture (once the powder is hydrated with sterile water) detects a lower number of CFU than on the surface of agar [10] [11]. The two DSU® epidemiology surveillance tools for *S. aureus* are presented as a feasibility study and to generate basic data to substantiate its feasibility.

2. Materials and Methods

2.1. Quantitative Analysis with Pure and Anterior Nares Cultures: EPI-M® and aureusAlert®

1) Pure Cultures:

ATCC 43300 (MRSA), ATCC 33591 (MRSA), ATCC 25923 (MSSA), ATCC 29213 (MSSA), ATCC 12228 (*S. epidermidis*), MRSA USA 600 (6 isolates), MRSA USA 300 (2 isolates), and MRSA USA 100 (2 isolates), plus 5 clinical patient laboratory isolates of MRSA and 5 of MSSA were utilized.

A suspension of each of the bacterial isolates was made in normal physiological saline to a 0.5 McFarland standard. From this suspension, 0.1 mL was transferred to 9.9 mL of sterile normal saline and vortexed well. Using a quantitative pipette (Rainin, Rainin Instrument LLC, Oakland, CA) 1 and 10 microliters of the suspension was transferred to aureusAlert® and EPI-M® and a dilution series generated. Colony counts were made from the 0.5 McFarland standard and the 9.9 mL normal saline [8].

2) Human surveillance study:

Nasal swabs (Culturette™ II, Becton, Dickinson and Company, Cockeysville, MD) were obtained from people entering or leaving a building at the following locations: Yale-New Haven Hospital (New Haven, CT, USA), Walter E. Washington Convention Center (Washington, D.C., DC, USA), George Washington University Hospital (Washington, D.C., DC, USA) and Tampa General Hospital (Tampa, FL, USA) to mimic a random selection of community inhabitants. Two swabs were obtained from the same nostril. There was no subject information collected nor were they made aware of the results.

2.2. Conventional Procedure

S. aureus: Volunteer nasal swab specimens were aseptically transferred to Trypticase Soy Broth (TSB) containing 6.5% NaCl for 24 hours enrichment at 33˚C - 35˚C. The enrichment samples were subcultured on to Tryptic Soy Sheep Blood Agar (TSBA) plates, and the plates were incubated aerobically for 24 hours at 35˚C. Suspicious colonies of *S. aureus* were identified by using standard laboratory methods including catalase, tube or slide coagulase, Gram staining, and mannitol salt agar. Confirmed *S. aureus* colonies were tested for methicillin resistance using the CLSI recommended reference method (e.g., 30 mcg cefoxitin disk) [12]. In addition, an assay for PBP-2 (Remel, Lenexa, KS) was performed.

MRSA: Cultures were inoculated into TSB with 6.5% NaCl and incubated for 18 - 24 hours. A subculture was

made to blood agar (BA) plates and a Staphaurex® (Remel, Lenexa, KS) test performed. In addition, an assay for PBP-2 (Remel, Lenexa, KS) was performed. Antibiotic susceptibility test (AST) was performed by both an agar dilution method (Mueller-Hinton agar with 4 mcg/mL oxacillin, Becton, Dickinson and Company, Cockeysville, MD) and also by the Sensitire™ (Trek Diagnostic Systems, Oakwood Village, OH) microdilution method.

2.3. Defined Substrate Utilization® Tools

All ingredients of the Defined Substrate Utilization® (DSU®) tools are optimized in powder form in a tube and are performed in the same way. Each tube is labeled with the name of the test, and a specific amount of water is transferred to the tube to hydrate the powder. The sample is added to the tube and incubated at 35˚C. A positive result, which can occur any time after the initiation of incubation, is seen as follows:

Interpretation: EPI-M®—the liquid changes color from straw-colored to amethyst with an increase in coalescence for a positive result; no change from straw-colored is a negative result. aureusAlert®—a clot or coalescence forms in the liquid for a positive result; no observable clot or coalescence is a negative result. Tubes are held a maximum of 24 hours before calling them negative (see **Figure 1**, **Figure 2**, **Figure 3** and **Figure 4**).

3. Results

Control *S. aureus* standards ATCC 25923 (MSSA) and ATCC 43300 (MRSA) plus 5 MSSA and 5 MRSA isolates from patients were tested. MSSA and MRSA isolates were diluted from 7 log10 to 1 log10 and incubated at 35˚C and 23˚C. Based on quantitative analysis of pure cultures, the Defined Substrate Utilization® (DSU®) tools were able to detect as low as 20 CFU (18 hours of incubation) with both MSSA and MRSA (**Table 1**, **Table 2**). **Table 3(a)** and **Table 3(b)** present results of the nasal screening of normal subject volunteers in aureusAlert®. **Table 4(a)** and **Table 4(b)** present the nasal screening results in EPI-M®. For both the detection of *S. aureus* and MRSA there was no difference between the DSU® tools and conventional methods. aureusAlert® and EPI-M® showed a specificity of 100%.

Figure 1. EPI-M® MRSA negative—straw-colored.

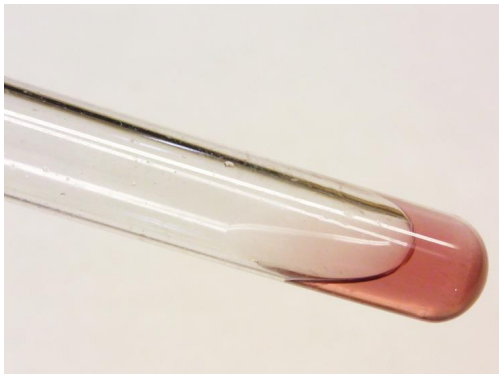

Figure 2. EPI-M® MRSA positive—amethyst.

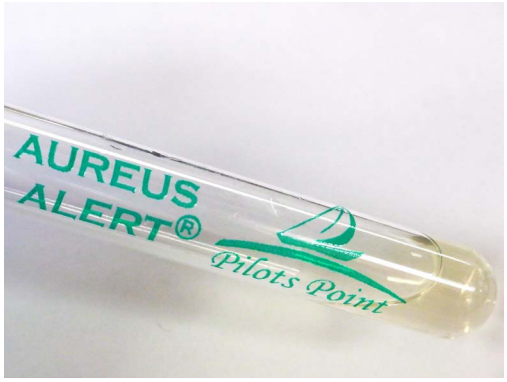

Figure 3. aureusAlert *S. aureus* negative—liquid.

Figure 4. aureusAlert *S. aureus* positive—clot.

Table 1. Detection time of quality control clone ATCC 25923 in aureusAlert®.

MSSA ATCC 25923 (CFU/mL)	Detection Time at 35˚C
7 log10	2.0 h
6 log10	3.0 h
5 log10	4.0 h
4 log10	7.0 h
3 log10	10.0 h
2 log10	15.0 h

Table 2. Detection time of quality control clone ATCC 43300 in EPI-M®.

MRSA ATCC 43300 (CFU/mL)	Detection Time at 35˚C
7 log10	2.0 h
6 log10	3.0 h
5 log10	4.0 h
4 log10	6.5 h
3 log10	14.0 h
2 log10	18.0 h

4. Discussion

Described here is a method to detect *S. aureus*, both MSSA and MRSA, directly from a human sample. Animal and environmental samples have not been analyzed. The key innovation was to optimize all ingredients and

Table 3. Detection comparison of *S. aureus* nasal swabs in aureusAlert® and conventional methods.

(a)

	Conventional Positive	Conventional Negative	Total
aureusAlert® Positive	450	20	470
aureusAlert® Negative	10	1787	1797
Total	460	1807	2267

(b)

Site	aureusAlert® (Positive/Negative)	Conventional (Positive/Negative)
Tampa General Hospital	69/322	72/322
Yale-New Haven Hospital	63/289	58/289

Table 4. Detection comparison of MRSA nasal swabs in EPI-M® and conventional methods.

(a)

	Conventional Positive	Conventional Negative	Total
EPI-M® Positive	49	2	51
EPI-M® Negative	1	480	481
Total	50	482	532

(b)

Site	EPI-M® (Positive/Negative)	Conventional (Positive/Negative)
Tampa General Hospital	33/317	32/317
Yale-New Haven Hospital	19/217	18/217

generate a stable, ready-to-use powder. The system's central ingredient is an enhanced coagulase substrate. The enhanced coagulase reaction overcame the observation by Selepak and Witebsky that rabbit plasma variability precluded the ability to directly detect *S. aureus* directly from samples. In one form, aureusAlert®, all *S. aureus* are detected. Using the aureusAlert® as a base, the addition of a mecA inducer specifically distinguishes MRSA (EPI-M®). By employing the definitive tests in the right milieu, as low as 20 CFU of MSSA and MRSA could be detected.

This study demonstrated that the sensitivity was equivalent to conventional methods by direct comparison and molecular methods from literature references. The time to detection was in the clinically useful range for the epidemiological screening of anterior nares. Because coagulase was chosen as the core detection system, a specificity of 49/49 for EPI-M® was seen from sampled subjects. Likewise, aureusAlert® being an enhanced coagulase test also showed complete agreement with conventional identifications.

The bacteria remain viable in the aureusAlert® and EPI-M® tools. The tubes can be transported, as is, from the field for further analysis (e.g., antibiotic susceptibility testing, molecular fingerprinting). The powder format offers great flexibility of use. It has the ability to gather information from a broad spectrum of sampling: in the field, in satellite facilities, in clinics, and in large volume central laboratories. It requires no skilled labor. Because it is a stable powder, it can be inexpensively transported. Its cost is 20% of genetic amplification and 75% of conventional processing surveillance samples. The feasibility of the method has been presented.

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