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Comparison of the novel Uroquattro HB&L™ system and classical phenotypic method for rapid screening of multidrug-resistant organism colonization at the University Hospital Centre Split, Croatia

Usporedba nove brze metode Uroquattro HB&L™ s klasičnom fenotipskom metodom probira kliconoštva višestruko-otpornim organizmima u Kliničkom bolničkom centru Split, Hrvatska

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Abstract

Background. Infections caused by multidrug-resistant organisms (MDRO) are difficult to treat and associated with poor outcomes for patients. Therefore, early identification and management of colonization are essential as first steps in infection prevention. Culture-based methods have been widely used for MDRO screening. The turnaround time (TAT) for the identification of carriers varies between 48-72 h with this method. The aim of our study was to compare the performance of the new rapid semiautomatic method for detection of MDRO (HB&L Uroquattro, Alifax) with standard cultivation on selective media.

Methods. Twenty-one axillary, 20 nose and 19 rectal swabs were taken in duplicate on two selected days at the University Hospital Centre Split, Croatia. Swabs were cultivated and MDRO isolates were identified on selective media (Chromagar MRSA and Chromagar ESBL) according to the standard operating procedure. Novel Alifax method was performed according to manufacturer's instructions.

Results. TAT for the new method was significantly lower (6.5 h) in comparison to the classical method. With classical method, 10 extended spectrum ß-lactamases (ESBL) producing strains from 10 different rectal specimens were isolated on Chromagar ESBL media. Exactly the same specimens were positive for the presence of ESBL-producing bacteria by rapid Alifax method. On selective Chromagar MRSA media, 11 MDRO were isolated, while rapid method detected 11 MDRO from the same specimens.

Conclusions. High concordance of positive and negative results obtained with classical and rapid method is encouraging. However, our study was performed on a small sample size and further research with larger sample size is needed.

Sažetak

Uvod: Infekcije uzrokovane višestruko otpornim organizmima (MDRO) se teško liječe i često imaju nepovoljan ishod za bolesnika. Budući da infekciji najčešće prethodi kolonizacija, rana identifikacija koloniziranih bolesnika je neophodna u prevenciji nastanka infekcije. Za probir MDRO najčešće se koriste kultivacijske metode kojima vrijeme potrebno za izdavanje nalaza (TAT) iznosi od 48 do 72 h.

Cilj ove studije bio je usporediti rezultate nove, brze, poluautomatske metode za detekciju MDRO (HB&L Uroquattro, Alifax) sa standardnom kultivacijom na selektivnim podlogama.

Metode/materijali: Tijekom dva nasumično odabrana dana, bolesnicima hospitaliziranima u Kliničkom bolničkom centru Split, uzorkovano je ukupno 21 aksilarnih, 19 rektalnih te 20 briseva vestibuluma nosa. Svaki obrisak je prikupljen u duplikatu, jedan za kultivacijsku, a drugi za brzu poluatomatsku metodu. Brisevi su kultivirani i MDRO izolati identificirani na selektivnim podlogama (Chromagar MRSA i Chromagar ESBL) prema standardnom laboratorijskom protokolu. Nova Alifaxova metoda je provedena prema uputama proizvođača.

Rezultati: U usporedbi s klasičnom metodom, TAT nove metode je bio značajno kraći (6,5 h). Klasičnom metodom je na Chromagar ESBL selektivnom mediju izolirano 10 sojeva koji stvaraju ß-laktamaze proširenog

spektra (ESBL) iz 10 različitih rektalnih uzoraka. Isti uzorci su bili pozitivni na prisustvo ESBL- producirajućih bakterija brzom Alifaxovom metodom. Kultivacijom na selektivnom Chromagar MRSA mediju i brzom metodom otkriveno je 11 uzoraka pozitivnih na prisustvo MDRO.

Zaključak: Visoki stupanj podudarnosti otkrivanja pozitivnih i negativnih uzoraka dobiven klasičnom i novom metodom, kao i značajno skraćivanje TAT-a novom metodom je ohrabrujući. Budući je studija provedena na relativno malom uzorku, potrebno je istraživanje proširiti na veći broj ispitanika.

Introduction

High rate of antimicrobial resistance (AMR) to multiple agents has been observed among Gram-negative and Gram-positive bacteria worldwide and represents a serious healthcare concern on the local and national level. Multidrug resistance (MDR) is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Infections caused by these highly resistant bacteria are associated with higher morbidity and mortality rates, can lead to inadequate or delayed antimicrobial therapy and are associated with poorer patient outcomes[1].

The European Centre for Disease Prevention and Control (ECDC) has estimated that infections caused by multidrug resistant organisms (MDRO) are responsible for about 25 000 deaths in Europe annually[2].

Among clinically significant MDRO are methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producing Gram-negative bacilli such as *Escherichia coli* and *Klebsiella pneumoniae*[3].

Methicillin resistance in *S. aureus* is the result of a decreased binding affinity of methicillin to its target, penicillin binding protein 2 (PBP2), owing to acquisition of an altered PBP2 (PBP2a) encoded by the *mecA* gene harboured on a mobile genetic element, the staphylococcal chromosome cassette *mec* (SCC*mec*). Even though MRSA has showed a decreasing trend during the last decade, it is still important nosocomial pathogen and a matter of greatest importance for public health[4,5]. *S. aureus*, including MRSA, is a common colonizer of the nares. The absence of MRSA nares colonization has reported to be the negative predictor of MRSA pulmonary infections, specifically pneumonia. Nasal surveillance of MRSA colonization is used for infection control and prevention purposes. However, recent literature has highlighted MRSA nasal screening as a useful antimicrobial stewardship screening test for avoiding unnecessary empiric MRSA therapy, including vancomycin[6].

Multidrug-resistant Gram-negative bacilli are emerging as a major challenge to human health, especially in low- and middle-income countries[7]. Resistance to broad-spectrum antibiotics is commonly observed in *Enterobacterales* family and may occur by different mechanisms, including permeability defects and efflux overproduction. However, the production of extended-spectrum β-lactamases (ESBL), broad spectrum β-lactamases which are able to hydrolyse expanded-spectrum β-lactam antibiotics, is the most significant mechanism and was first described in 1985 in a *Klebsiella pneumoniae* strain[8]. By the end of the decade, a broad range of bacteria producing these enzymes have been identified in healthcare facilities worldwide. Today, ESBL is one of the most common resistance mechanisms of Gram-negative bacteria and ESBL-producing bacteria represent one of the most important groups of nosocomial pathogens[9,10]. Moreover, high colonization rates among hospitalized and non-hospitalized individuals have been detected[11,12]. One suggested risk factor for the development of ESBL infection is gut colonization with these organisms. Karanika and colleagues in their meta-analysis of 66 studies report a global prevalence of ESBL faecal colonization of 14%. A recent cohort study performed by Cornejo-Juarez and colleagues in patients with hematologic malignancies revealed that patients with faecal ESBL colonization were 3.5 times more likely to develop ESBL bacteraemia compared with those not colonized[13,14].

High prevalence of MDRO among patients in intensive care units (ICU) raises particular concerns that require improved prevention and control strategies. Vertical approaches are mainly based on screening and contact precautions and/or decolonization of MDRO carriers. On the other hand, horizontal strategies include standard precautions (i.e., hand hygiene), universal decolonization, antimicrobial stewardship, and environmental cleaning[15].

One goal of this strategy is to identify patients who are colonized with a MDRO as early as possible. This aligns with recommendations to screen those with a high risk of being colonized with an MDRO, like ICU patients[16]. Admission screening is an effective component of infection control interventions to control ESBL outbreaks and is recommended in several guidelines for the control of drug resistant Gram-positive organisms such MRSA[17]. Screening for MRSA colonization at admission using culture-based approaches requires 24 to 72 hours until the results are available on the wards. During this time MRSA can spread among inpatients. Köck and colleagues concluded that

screening can help decrease MRSA infection rates in hospitals. The provided evidence support the introduction of MRSA active surveillance programme in hyperendemic MRSA settings due to cross-infections in spite of the high level of compliance with standard precautions[12]. For the detection of ESBL, phenotypic and genotypic methods are employed. Phenotypic approaches are based on the detection of synergy between β-lactam agents and specific substances that inhibit each enzyme type. Genotypic methods, which include polymerase chain reaction (PCR) and next generation sequencing (NGS) allow for a highly accurate characterization of resistance mechanisms but are available only in well-equipped facilities with high laboratory budget[18].

The aim of our study was to evaluate the ability of the new rapid, semiautomated liquid-culture method to detect ESBL-producing *Enterobacterales* and MRSA in clinical swabs from potentially colonized subjects, and to compare it to classical method.

Materials and methods

On two selected days (September 2017), screening of MRSA and ESBL-producing *Enterobacterales* colonization was performed on intensive care unit patients at the University Hospital Centre Split, Croatia. In total, 60 clinical specimens (21 axillary's swabs, 20 swabs and 19 rectal swabs) were collected. Specimens were obtained (with Copan swabs, Brescia, Italy) in duplicate, one for rapid and the other one for classical MDRO colonization screening.

Classical method was performed by cultivation on solid and liquid selective media. Chromagar MRSA (Chromagar, Paris, France), chromogenic medium for the isolation and identification of methicillin resistant *Staphylococcus aureus* and Chromagar ESBL (Chromagar, Paris, France), chromogenic medium for overnight detection of Gram-negative bacteria producing extended spectrum β-lactamase, were used. These media contain a mixture of antibiotics and chromogenic substrates that helps rapid identification of ESBL-producing *Enterobacterales* and MRSA species, based on the specific colour appearance. Nose and axillary's swabs were inoculated on MRSA Chromagar, while rectal swabs were inoculated on ESBL Chromagar. Inoculated plates were incubated aerobically at 35 °C for 48 h. Also, thioglycollate broth (Liofilchem, Roseto d. Abruzzi, Italy) was used for the overnight enrichment. On the second day, all liquid media were subcultivated on Chromagar MRSA and Chromagar ESBL selective media and incubated for another 24h. Presumptive colonies of *Enterobacterales* and MRSA, from primary subcultivation plates, were tested for identification and antibiotic susceptibility by using VITEK 2 system (bioMérieux, Marcy l´Etoile, France). GP, GN, AST–N233, and AST-XN05 VITEK cards were used. Methicillin resistance was confirmed by inducing cefoxitin (Mastdiscs AST, Merseyside, UK) disk (30 μg), while ESBL-production was confirmed by double-disc synergy testing (Mastdiscs Combi ESBL ceftazidime paired ID discs and Mastdiscs Combi ESBL cefotaxime paired ID discs, Merseyside, UK) on Mueller–Hinton agar (Liofilchem, Roseto d. Abruzzi, Italy). Plates were incubated at 35°C for 24h. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretation criteria were applied on all antimicrobial testing[19].

Rapid screening method, based on laser light scattering technology, was performed by Uroquattro HB&L™ system (Alifax, Padova, Italy). For that purpose, HB&L MRSA (SI 1001.900) and HB&L ESBL/AmpC (SI 1001.930) kits were used. The screening was performed according to the manufacturer's instructions. Briefly, Alifax culture vials were inoculated by dipping the swabs into the culture broth for 10-15 minutes. After that, swabs were removed. Then, regenerated supplement (from MRSA or ESBL/AmpC kit) was added to the inoculated Alifax vials. The vials were placed in the Uroquattro HB&L™ system and incubated for 6.5 h at 36 °C. The growth phases of bacteria were monitored, providing real time growth curves. The samples positive to HB&L™ MRSA kit detect the presence of Gram-positive bacteria with resistance to methicillin/oxacillin/cefoxitin. The positive samples to HB&L™ ESBL/AmpC kit detect the presence of Gram-negative bacteria with resistance to cephalosporin.

Results obtained with HB&L™ MRSA and ESBL/AmpC kits were compared to classical routine method.

Results

In total, 40 specimens were screened for MRSA colonization while 20 specimens were screened for ESBL colonization. Of the total of 40 specimens screened for MRSA, 2 (5.00 %) were positive. On Chromagar MRSA media, besides 2 MRSA strains, another 9 resistant strains were isolated. They were identified as coagulase negative methicillin resistant *Staphylococcus sp.* (4 strains) and MDR *Acinetobacter baumannii* (5 strains). At the end of incubation time of Alifax vials, 10 positive samples were detected. They completely matched the positive results of classical method, and the strains identification was identical.

Of the total of 20 specimens screened for ESBL, 10 (50.00%) were positive. With conventional method, 10 positive ESBL stains from 10 different rectal specimens

were isolated on Cromagar ESBL media: seven isolates of *Klebsiella pneumoniae*, one *Klebsiella oxytoca*, one *Proteus mirabilis* and one *Providencia stuartii*.

Same 10 rectal specimens were positive for ESBL growth by rapid Alifax method after the analysis time passed.

TAT for Alifax method was shorter than classical method because incubation time took only 6.5 h, while classical method incubation lasted from 24 (primary plates) to 72 h (subcultivation after broth enrichment).

Discussion

Antibiotic resistance is a serious threat to public health in Europe, leading to prolonged hospital stays, treatment failures and deaths. The most concerning antimicrobial resistance trends in Europe are related to the occurrence of resistance in Gram-negative bacteria (e.g. *Escherichia coli, K. pneumoniae*). Many of the isolates are ESBL-positive and show resistance to additional antimicrobial groups.

Methicillin resistance in *Staphylococcus aureus* shows decreased trend, but less pronounced compared to the previous years[20].

Strategy directed at interruption of cross-transmission is considered the most cost-effective in managing infections caused with MDRO.

The first steps in infection prevention are early identification and management of colonization, including contact precautions measures, hand and environmental hygiene and antimicrobial stewardship. Many methods for screening and identification of MDRO have been proposed. Phenotypic approaches require overnight incubation and this delay in identification is the main disadvantage of culture-based approaches, such as the double-disk synergy test, three-dimensional tests, gradient diffusion tests as well as automated systems. Genotypic methods are highly accurate in characterization of resistance mechanisms and being of great advantage in cases where phenotypic results are unclear. However, these methods must often translate into high costs, thus limiting the availability of such methods in routine laboratories (18)(15). Therefore, fast and reliable method for rapid screening and identification of MDRO is most needed. Here, we compared new semiautomatic Alifax method for MDRO screening with routine cultivation on selective media. The results with these two methods were completely identical, but new method had shorter TAT because of shorter incubation time (6.5h vs. 24-72h). Alifax method has some limitation. According to manufacturer's instructions, all positive samples should be further tested with confirmatory tests (for methicillin resistance or for ESBL- production, depending of used kits). Some glucose non-fermenter Gram-negative bacteria (e.g. *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) can be resistant to cephalosporin used in Alifax kit and therefore give positive results despite not being ESBL/AmpC-producing *Enterobacteriales* spp. Similarly, some Gram-positive bacteria (e.g. *Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus haemolyticus*) can be resistant to cefoxitin despite not being MRSA strain.

Conclusions

High concordance of results obtained with classical and rapid screening method is encouraging. According to our findings, novel method is fast and reliable and it can be recommended for a routine MDRO screening. Isolation precaution measures for colonized patients can be implemented on the same day. Additionally, pre-emptive isolation precaution measures after patient transfer from other hospital, long-term care facility or high risk ward, can be terminated earlier. However, these results should be confirmed on a bigger sample size.

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Conflict of interest

Nothing to declare.

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