Several virulence factors, including those identified in cerebrospinal fluid, have sequences and analyzers for the 16S rRNA genes.

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Abstract

A set of broad-range PCR primers for the 16S rRNA gene in bacteria, as well as three series of oligonucleotide probes to detect the PCR product, were examined. The first set of probes covers a wide spectrum of bacteria and includes a universal bacterial probe, a gram-positive probe, a Bacteroides-Flavobacterium probe, and two probes for various gram-negative species. The second series was created to detect PCR products from seven of the most common bacterial species or groups that cause meningitis: *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. agalactiae, Escherichia coli* and other enteric bacteria, Listeria monocytogenes, and *Staphylococcus aureus*. The third series was created to detect DNA from species or genera that are widely thought to be potential contaminants in clinical samples, such as cerebrospinal fluid. The pathogen and contaminant probe sequences from CSF were compared to existing sequence information and sequencing data from 32 distinct species. The CSF pathogen and contaminant probes were tested against DNA from over 60 different strains, and with the exception of the coagulase-negative *staphylococcus* probes, these probes correctly identified bacterial species known to be prevalent in CSF.

Keywords: *Staphylococcus* probes, Cerebrospinal fluid, DNA.

Introduction

Three runs were performed successively to detect each organism separately. As species-specific targets, the ctrA gene of *Neisseria meningitidis*, the bexA gene of *Haemophilus influenzae*, and the lytA gene of *Streptococcus pneumoniae* were employed [1]. The sequences of the primers for each run, sample DNA was mixed with 2x QuantiTect SYBR Green PCR master mix (QIAGEN Inc., Valencia, CA) containing a buffer, dNTP mix, $MgCl_2$ and HotStarTaq DNA polymerase, the primer, and RNase-free water for a total volume of 25 L. The following temperature programme was used to amplify DNA with the step one Real-Time PCR machine (Applied Biosystems): an initial Hot start Taq activation step at 95°C, followed by an initial denaturing step at 95°C and 40 PCR cycles of denaturing at 95°C, annealing at 50°C, and extension at 72°C, followed by melting curve stage of 95°C and 60°C.

Pathogen epidemiological survey in cerebrospinal fluid

Rapid and precise laboratory diagnoses continue to be an important step in the ultimate diagnosis of bacterial meningitis, determining treatment and adopting preventative measures for close contacts where required. Conventional culture methods, despite being the gold standard diagnostic technique, cannot be relied on in certain situations due to the delay in results availability and the relatively low sensitivity that has been repeatedly reported globally, as well as in conditions where prior antimicrobial therapy has been received. Previously, a large percentage of culture-negative samples were recorded in Egypt [2]. These findings were explained by the fact that most patients got antimicrobial drugs that can be acquired over-the-counter even before clinical examination, as well as the occasional delay in CSF sampling. For the reasons stated above, a molecular method (real-time PCR) in a study to improve the diagnosis of bacterial meningitis in culture-negative purulent CSF samples. CSF culture is documented to be positive only in 1/10th of the previously antibiotic treated patients in developing countries. Same result was found, who reported low rates of culture positive CSF samples (8%) of suspected cases with bacterial meningitis. This low yield in culture results could be attributed to the fact that antimicrobials are being dispensed without prescriptions in Egypt [3].

Virulence factors

Total of 13 virulence factors (VFs) encoded by chromosome or plasmid, were investigated. The genes included the PAI marker, the adhesin genes (papG, papC, sfaS), the toxin genes (hlyA, cnf1), the capsule synthesis gene (kpsII), the siderophore genes (fyuA, iroN, iutA), the serum resistance genes (iss and traT), the brain microvascular endothelium invasion gene (ibeA), and the outer membrane protein gene.

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Phylogenetic classification based on 16S rRNA sequences

TrimAI analysis was performed after nucleotide BLAST analysis. The sequences of all 16S rRNA genes have been deposited in the NCBI GenBank database. The sequence similarities were analysed using the web-based SINA alignment service with the following criteria: discard sequences having less than 90% identity to the consensus *E. coli* sequence and sequences longer than 700 nt. MUSCLE was used to achieve multiple sequence alignment of 16S rRNA partial sequences [4, 5].

Conclusion

The detection of virulence factor genes in non-pathogenic phylogenetic groups A and B1 signified that the presence of the virulence genes might not be directly correlated to the phylogenetic group. Moreover, the high detection of serum resistance factors might be a prerequisite virulence factor for bacteria that cause systemic bloodstream infections. Also, yersinabactin could be a characteristic virulence factor for the bacteraemia *E. coli* isolates. Moreover, ExPEC isolates belonging to the same phylogenetic group can have variable resistance and virulence properties

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