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Mini Review

Distribution and evolution of virulence factors in septicemic *Escherichia coli*

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ABSTRACT

Bacterial septicemia is an emerging clinical problem which is increasing in significance due to the rapid spread of antibiotic resistance. In order to combat this problem, it is essential to identify the critical virulence factors of these septicemic strains and study their functions and role in pathogenesis. Here, we survey some of the virulence factors which are essential for sepsis and are potential candidates for development of new drugs or vaccines.

Introduction

Most of the *Escherichia coli* strains are commensal but there are also many pathogenic strains. These can be divided into intestinal pathogens and extraintestinal pathogens (ExPEC = extraintestinal pathogenic *E. coli*). This last group is involved in a large variety of diseases. The most common ones are the urinary tract infections (UTI) that may develop into pyeolonephritis, kidney failure, and productivity loss. UTIs are responsible for more than 7 millions patient visits and one million hospital admissions (due to complications) per year in the United States only. Additional diseases include neonatal meningitis (NBM) responsible for about 0.25 per 1000 live births in industrialized countries and 2.66 per 1000 in developing countries. ExPEC strains are also common in intraabdominal infections, respiratory tract infections, wound and surgical infections (Johnson and Russo, 2002a,b; Johnson et al., 2004, 2005; Smith et al., 2007).

E. coli pathogens were not considered important in terms of infectious diseases. However, in the 20th century, the situation changed because of the introduction of antibiotics. Antibiotics almost eliminated many infectious diseases (the plague, typhoid fever, cholera) and created a shift to infections by bacteria that are less virulent but more resistant to antibiotics – such as *E. coli* (Mylotte et al., 2000; Stoll et al., 2002; Pitout et al., 2005; Johansen et al., 2006; Vicente et al., 2006; Zemkova et al., 2007; Marchaim et al., 2008).

One of the most serious syndromes caused by ExPEC is colisepticemia (or colibacillosis). This disease is usually secondary to UTI and is very common in institution-acquired infections. *E. coli* is a leading cause of bloodstream infections in nursing homes (Mylotte et al., 2002), hospitalized persons (Siegman-Igra et al., 2002), and children, especially newborns (Milch et al., 1977; Angus and Wax, 2001; Kim et al., 2002; Stoll et al., 2002; Kapoor et al., 2005; Melzer and Petersen, 2007; Ortega et al., 2007; Bizzarro et al., 2008; Marchaim et al., 2008). Colisepticemia is responsible for over 40% of the bacteremia cases in community- and hospital-acquired infections and is the major causes of mortality (more than 80%) from these infections. It is also the main cause of mortality in immunosuppressed patients (HIV, chemotherapy, old age) (Mylotte, 2005; Johansen et al., 2006; Cheong et al., 2007; Wyllie et al., 2007; Laupland et al., 2008; Marschall et al., 2008; Skogberg et al., 2008).

Today sepsis kills more people than lung cancer and more people than bowel and breast cancer together. The economic burden of sepsis is significant and according to the estimates comes to around 7.6 billion euros and US$16 billion per year in Europe and US, respectively (Angus et al., 2001; Angus and Wax, 2001; Robson and Daniel, 2008).

The high resistance of ExPEC strains to antibiotics suggests that vaccination would be a potential solution. However, ExPEC include a very large number of serotypes which do not cross-react. Therefore, it is not possible to develop a "simple" vaccine (inactivated bacteria, etc.), and novel approaches have to be used. In order to develop a successful immunization system or to identify new potential drug targets, it is essential to define the factors responsible for the virulence of ExPEC strains.

What is required to cause a systemic infection?

There are three major categories of factors that are essential for septicemia:

- Survival in serum – serum is bactericidal, mainly because of the activity of the complement.

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- Efficient iron uptake – the mammalian tissues and blood are depleted of free iron, as all the iron is tightly-bound to proteins such as transferrin. In order to survive in the host, bacteria have to code for one or more efficient iron binding systems.
- Internalization by host cells – this is not absolutely obligatory, but is still important for virulence and for protection from phagocytosis.

Genomic approach for identifying virulence factors of septicemic strains

The best procedure for identifying unique genes, present only in septicemic strains, is by whole-genome sequencing. Alternative methods include the molecular procedure of suppression subtractive hybridization (SSH). This procedure allows the comparison of two genomes, and detection of specific sequences that are present exclusively in one of them. This technique has been shown to be extremely efficient in detecting genomic sequences and genomic islands that are strain-specific and was used to identify virulence factors by comparing pathogenic and non-pathogenic bacterial strains of the same species (Akopyants et al., 1998; Walker and Verma, 2002; Zhang et al., 2000; Janke et al., 2001). Subtractive hybridization experiments comparing *E. coli* K-12 with two septicemic *E. coli* strains – one of serotype O2 and the other one of serotoype O78 – resulted in the identification of about 160 unique sequences that were verified by PCR (Mokady et al., 2005a,b).

The unique sequences include a high ratio of virulence-related genes, or genes with no blast homology, which could also be virulence-related. The presence of numerous mobility-associated sequences, such as transposases and integrases, along with phage-related sequences, plasmid sequences, and insertion sequences-associated sequences indicates the presence of genomic areas that were acquired horizontally, potentially related to virulence. The prevalence of sequences associated with genomic plasticity in the subtractive libraries supports the assumption that the pathogenic strains evolved by processes involving genome remodeling and horizontal acquisition of genomic regions from other pathogenic bacteria.

Distribution of the specific sequences among the septicemic strains

Among the sequences unique to strains O2 and O78 (Mokady et al., 2005a,b) about 45 appeared to be associated with virulence. A large number of septicemic strains were then screened to determine which of the unique sequences is present in many (or all?) of them. The results indicated that most of the unique sequences were patchily distributed, most of them only present in a small subset of strains, and may have therefore been acquired by fairly recent horizontal gene transfer. A few genes probably represent an ancient transfer event, as they are found in all the pathogenic strains tested, but not in K-12. These probably originate from genes that are essential for sepsis.

The fact that not many virulence factors are common in all the septicemic strains examined is unexpected, since phenotypically, all the strains cause the same disease. The findings imply that the various strains are using different factors with similar roles in the various stages of the infection process. Thus, each step in the infection process can be mediated by a number of alternative virulence factors, and each strain may have a unique combination of such factors. This assortment of virulence genes is apparently made possible by the variety of genetic factors contributing to genome plasticity, such as plasmids, phages, and transposable elements. The fact that extant *E. coli* strains vary so much in the content of their genomes indicates that this "mix and match" combinatorial approach has been a successful evolutionary strategy for these species, which can colonize many different tissues and hosts.

Previous genomic comparisons of *E. coli* strains have shown great differences in gene content. However, none of the comparative genomics of sequenced *E. coli* strains compared strains, which cause the same disease and target the same host tissues. The only exception is in the 2-genome sequences of O157:H7. In these strains, the virulence factors and genes coding for them show a very high degree of similarity (Perna et al., 2001; Jin et al., 2002). These results are in contrast to our findings in the septicemic O2 and O78 serogroups, showing a very high level of genome plasticity.

Essential virulence factors

Although most of the sequences unique to septicemic strains have appeared in only a few strains, there are several genes, which are present in all the strains, in one form or another. These common virulence factors are presumably essential for the infection process. Here we outline them briefly.

ColV plasmids

Many of these factors are coded on a plasmid – ColV – that is present in the majority of septicemic strains. The ColV plasmids carry genes encoding the aerobactin iron uptake system as well as genes coding for serum resistance (Warner et al., 1981; Valvano and Crosa, 1984; Valvano et al., 1986; Waters and Crosa, 1986; Zgur-Bertok et al., 1990; Waters and Crosa, 1991; Gophna et al., 2003a). These plasmids vary in size but are usually conjugative and often carry genes for antibiotic resistance.

Iron acquisition systems

Iron is an essential cofactor for bacterial metabolism. The fact that iron in body fluids (e.g. plasma) is bound to host proteins such as transferrin, reduces its availability to far below concentrations allowing bacterial survival. Septicemic *E. coli* strains cope with the scarcity of iron by producing low molecular weight compounds (siderophores) which bind iron with high affinity and compete efficiently with host proteins. Once iron-bound, these chelators are taken up into the bacteria by specific receptors on their membranes.

Septicemic bacteria carry a variety of genes coding for iron uptake systems. As for other genes unique for septicemic strains, not all the strains carry all the iron uptake systems, but every strain carries at least several. The most common one is Aerobactin whose presence is correlated with virulence and is usually located on the ColV plasmid (Warner et al., 1981; Valvano and Crosa, 1984; Valvano et al., 1986; Waters and Crosa, 1986; Lafont et al., 1987; Zgur-Bertok et al., 1990; Dozois and Curtiss, 1999; Gophna et al., 2003a).

Another iron uptake system which is abundant in septicemic strains is similar to that located on the HPI (high pathogenicity island) typical of pathogenic *Yersinia*. The HPI-encoded iron uptake system of septicemic strains includes genes for the biosynthesis of the siderophore yersiniabactin and genes coding for its receptor (Schubert et al., 1998; Karch et al., 1999; Gophna et al., 2001c; Janben et al., 2001).

Additional iron uptake systems found in several septicemic strains include the IroN system, the hemin receptor ChuA, and the *sitABCD* system (Negre et al., 2004; Rodriguez-Siek et al., 2005).

Serum resistance

Serum is bactericidal due to the activity of complement and additional inhibitory proteins. The molecular mechanism of serum resistance is not clear. Several factors potentially involved in serum