Emerging Resistance Among Gram-Negative Pathogens: State of the Challenge

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In the 1980s, antimicrobial resistance among gram-negative (Gm) pathogens seemed to be under control, with the availability of a growing anti-Gm armamentarium, including oxyimino-cephalosporins, fluoroquinolones, and carbapenems. Multiple treatment options were available in most infections and, while resistance to older antibiotics was common, it was caused by a relatively few stable mechanisms—TEM-1 β-lactamase, for example, accounted for over 90% of ampicillin resistance in *E. coli*. What is more, the residents of India and China—a third of the world's population—had yet to experience large-scale exposure to modern medicine, modern antibiotics, and their consequences.

Much has since changed, and physicians are now facing unprecedented clinical challenges due to the growing proliferation of multidrug-resistant Gm pathogens. David Livermore, PhD, Antibiotic Resistance Monitoring and Reference Laboratory, London, United Kingdom (UK), described current epidemiological, mechanistic, and demographic trends that are related to this worldwide epidemic.

E. coli is a crucially important Gm pathogen, causing 80% of urinary tract infections, and is the most frequent Gm agent of bacteremia. For 20 years, *E. coli* was among the most susceptible Gm bacteria to modern antibiotics, but, over the past decade it has joined *Enterobacter*, *Klebsiella*, and *Pseudomonas*. According to recent data from the European Antimicrobial Resistance Surveillance Network (EARS-net), increasing proportions of *E. coli* bloodstream isolates are nonsusceptible to fluoroquinolones and cephalosporins. In Italy, for example, rates of fluoroquinolone resistance among *E. coli* bloodstream isolates jumped from between 1% and 5% to between 25% and 50% from 2001 to 2009 [http://www.ecdc.europa.eu]. These Southern European resistance rates pale in comparison to those observed in South and East Asia. According to a 2007 survey, *E. coli* isolates from intraabdominal infections in China and India, 50% and 80% respectively, carried extended-spectrum β-lactamases (ESBLs), rendering them resistant to modern oxyimino-generation cephalosporins [Hawser SP et al. *AAC* 2009]. It is striking that resistance to third-generation cephalosporins among *E. coli* in India and China is now more prevalent than ampicillin resistance in *E. coli* in Sweden or Norway—countries that are known for their low antibiotic use and resistance.

The accumulation of resistance to fluoroquinolones and cephalosporins in *E. coli* and related species is clinically important, and is dramatically supported by a 2007 meta-analysis by Schwaber and Carmeli [*JAC* 2007], who found that patients with bacteremia due to ESBL-containing pathogens had increased mortality rates compared with those with non-ESBL strains (pooled RR, 1.85; 95% CI, 1.39 to 2.47; p<0.001). The difference was attributed to delayed effective treatment, with many of the ESBL-producing strains resistant to the physician's choice of empiric therapy. Early use of carbapenems, which are stable to ESBLs, might seem a logical solution to this problem; however, diverse carbapenemases are starting to emerge among Gms [Patel G et al. *Expert Rev Anti Infect Ther* 2011]. These include the VIM and NDM metallo and the KPC and OXA-48 nonmetallo types.

EARS-net data reveal that although most European countries still have very low rates of carbapenem nonsusceptibility (<1% in *Klebsiella* bacteremia isolates across the continent), resistance is widespread in Greece, where rates among *Klebsiella* species reached 40% in 2005 and 50% by 2009. A major shift in carbapenemase type also occurred in Greece. The initial problem was the spread of plasmids that encoded VIM metallo-β-lactamases among *Klebsiella* strains, but this has now been supplanted by the problem of a single *Klebsiella* strain with a nonmetallo 'KPC' carbapenemase that is spreading nationally [http://www.ecdc.europa.eu; Vatopoulos A *Eurosurv* 2008; Giakkoupi P et al. *Eurosurv* 2009]. Outbreaks of *Klebsiella* that produce the KPC carbapenemase were first noted in the United States (US) in 2005, but in addition to dissemination in the US, Israel and Greece, there are now growing clusters elsewhere in Europe, South America, and East Asia [Bratu S et al. *Arch Int Med* 2005; Nordmann P et al. *Lancet ID* 2009].

Pathogens that produce another carbapenemase, OXA-48, are resistant to carbapenems but are susceptible to cephalosporins (unless they also have ESBLs), a pattern that is not always recognized by automated systems and therefore often missed. OXA-48 carbapenemase has spread from Turkey, where it has been recorded since 2000, into North Africa and Europe, while similar enzymes have been recorded in India and South America [Benouda et al. *Ann Trop Med Para* 2010; Moquet et al. *EID* 2011; Cuzon et al. *IJAA* 2010; Cuzon et al. *AAC* 2008; Cuzon et al. *AAC* 2011; Goern et al. *IJAA* 2011; Matar et al. *CMI* 2008; Carrer et al. *AAC* 2010; Carrer et al. *AAC* 2008; Poirel et al. *AAC* 2011; Castanheira et al. *AAC* 2011].

NDM-1 carbapenemase is prevalent in India and Pakistan and has repeatedly been exported to Europe, North

America, and Asia by people who have had contact with medical facilities in the Indian subcontinent [Kumarasamy KK et al. *Lancet ID* 2010]. NDM-1 is often coded by plasmids that can spread readily among bacteria and is commonly produced together with rRNA methylases that confer aminoglycoside resistance. Some Gm pathogens with NDM enzymes have near-complete resistance—susceptible only to colistin, with or without moderate susceptibility to fosfomycin and tigecycline [Kumarasamy KK et al. *Lancet ID* 2010]. The dissemination of NDM-1 in parts of India is striking, with one survey revealing a 5% to 7% prevalence among *Enterobacteriaceae* species in Mumbai [Deshpande P et al. *Clin Infect Dis* 2010].

Multiresistant bacteria carriage rates among healthy individuals is facilitating the spread and repeated introduction into hospitals, and is a critical subject of international interest. Separate studies have shown gastrointestinal tract colonization by ESBL *E. coli* in 13% of job applicants in Saudi Arabia [Kadar et al. *ICHE* 2007] and in nursing home residents in Northern Ireland (40%) and Italy (64%) [Rooney PJ et al. *JAC* 2009; March A et al. *CMI* 2010]. A prospective study in Sweden revealed that one quarter of previously uncolonized individuals became colonized with ESBL *E. coli* during travel, with the highest rates of colonization associated with travel to East Asia (29%) and India (88%) [Tangden T et al. *AAC* 2010]. NDM-carrying bacteria were shown to inhabit the gut of 27% of inpatients and 14% of outpatients in military hospitals in Pakistan [Perry et al. *JAC* 2011].

The molecular basis of proliferating resistance among Gm bacteria is more complex than previously thought. Potent bacterial strains, such as sequence type (ST) 131 *E. coli* serotype 025 [Uchida et al. *IJAA* 2010; Guenther S et al. *JAC* 2010] and ST258 *K. pneumoniae* [Woodford N et al. *FEMS Micro Revs* 2011], play a pivotal role. *E. coli* ST131 commonly hosts plasmids that encode CTX-M-15 ESBL and has been instrumental in their international dissemination, while ST258 *K. pneumoniae* is playing a similar role in the spread of KPC carbapenemases. However, ST131 *E. coli* may also carry other ESBL types, including CTX-M-3 (Belfast) and CTX-M-14 (Far East), or may have no ESBL.

Deeper analysis shows that the plasmids that carry β-lactamase genes are remarkably dynamic and are constantly in flux. The dominant *E. coli* ST131 variant in the UK ('Strain A') typically contains a complex CTX-M-15 plasmid, EK499, which is a fusion between two parent plasmids, one of which (pEK516) resembles, but is not identical to those that commonly host CTX-M-15 internationally [Woodford N et al. *AAC* 2009]. Moreover, although CTX-M-3—prevalent in *E. coli* ST131 from Belfast—differs from CTX-M-15 by only 1 amino acid, the plasmids that encode CTX-M-3 and CTX-M-15 are completely different. In the case of NDM, the encoding gene has transferred swiftly among plasmids that belong to diverse incompatibility groups, though the mechanism of transfer is unclear. Prof. Livermore suggests that this genetic fluidity among bacteria is perhaps "the finest but most disturbing evidence of evolution we shall ever see."

The emergence of carbapenem resistance is beginning to drive the use of nonconventional antibiotics, such as colistin, tigecycline, and fosfomycin, but none of these is ideal, and some resistance is beginning to emerge, even to colistin [Kontopoulou K et al. *JHI* 2010]. In addition, current pipeline agents represent incomplete solutions. Avibactam, a new β-lactamase inhibitor, inhibits KPC but not metallo-β-lactamases, and while CXA-201 (cephalosporin CXA101+tazobactam) evades ESBLs and has very good anti-*Pseudomonas* activity, it lacks activity against carbapenemase producers [Livermore DM et al. *AAC* 2011; Livermore DM. *JAC* 2010].

Aging populations, shifting world economies, and inadequate sanitation add to an already complex problem of emerging resistance. The elderly, who form a growing percentage of the population, are more vulnerable to infections, and economic growth in India and China is greatly increasing access to sophisticated medicine and modern antibiotics, often in settings where infection control and regulation of antibiotic use are weak. These are the perfect conditions to drive the selection of resistance, illustrated by the ESBL rates. In India, the lack of public health infrastructure is a tremendous concern. A recent study by the Health Board of the Delhi Municipal Council found that 18% of tap water samples were contaminated with fecal bacteria and that hundreds of millions lack access to a flush toilet [UN News Center 2010]. Improving sanitation here to prevent the spread of resistant bacteria is as important as it was in the US in the early twentieth-century in preventing the spread of classical infectious diseases.

Development of new anti-Gm antibiotics is urgently needed and hinges on overcoming three challenges. First, discovery of agents that are capable of permeating the Gm cell wall and evading efflux is a key scientific challenge. Second, the regulatory pathway needs to confirm efficacy and safety, but has become too complex and often fails to test antibiotics in the types of patients or settings where they are most likely to be used. The third, and key economic challenge, is the likely return of antibiotic development. Short-term agents for acute infection do not generate the long-term income. These and other challenges must be confronted in order to effectively halt the spread of resistant Gm pathogens.