REVIEW

Investigating colistin drug resistance: The role of high-throughput sequencing and bioinformatics [version 1; referees: awaiting peer review]

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Abstract

Bacterial infections involving antibiotic resistant gram-negative bacteria continue to increase and represent a major global public health concern. Resistance to antibiotics in these bacteria is mediated by chromosomal and/or acquired resistance mechanisms, these give rise to multi-drug resistant (MDR) or extensive drug resistant (XDR) bacterial strains. Most recently, a novel acquired plasmid mediated resistance mechanism to colistin, an antibiotic that had been set apart as the last resort antibiotic in the treatment of infections involving MDR and XDR gram-negative bacteria, has been reported. Plasmid mediated colistin resistant gram-negative bacteria have been described to be pan-drug resistant, implying a state devoid of alternative antibiotic therapeutic options. This review describes the evolution of antibiotic resistance to plasmid mediated colistin resistance, and discusses the potential role of high-throughput sequencing technologies, genomics and bioinformatics towards improving antibiotic resistance surveillance, the search for novel drug targets and precision antibiotic therapy focused at combating colistin resistance, and antimicrobial resistance as a whole.

Keywords

Antibiotic resistance, Colistin resistance, Pan-drug resistance, Gram negative bacteria, Genomics, Bioinformatics
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Introduction
In the recent past, old antibiotic classes previously deemed unfit for treatment of bacterial infections due to associated toxicity concerns have been recommended for treatment in this type of infection\cite{1,2}. This has been attributed to the emergence of resistance to the most recently considered last line antibiotics, the carbapenems\cite{1,2}. Carbapenem resistance has been documented in bacteria belonging to the Enterobacteriaceae family, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*\cite{1,2}. The adoption of the old antibiotic agent category in routine empirical treatment has witnessed the use of a number of antibiotics such as colistin\cite{1,2}.

Despite this reversion, gram-negative bacteria continue to undergo chromosomal mutations, which render their respective treatments virtually impossible and hence a major threat to global public health. The effects of these antibiotic resistance mutations are further exacerbated by horizontal transfer of antibiotic resistance genes in the same bacteria. As such, this paper explores the current documented trends of colistin resistance in several African settings. Additionally, it also describes the evolution of antibiotic resistance to plasmid mediated colistin resistance and the potential role of genomics and bioinformatics in precision antibiotic therapy targeted towards combating colistin resistance and antimicrobial resistance.

Colistin resistance trends in Africa
Data on the antimicrobial resistance burden, particularly colistin resistance, in Africa remains limited\cite{3}. In 2014, the World Health Organization reported that antimicrobial resistance surveillance in Africa was a particularly difficult feat due to the scarcity of viable medical data, statistical information and unreliable laboratory capacity\cite{3}. Despite this, African countries remain un-exempted from this worldwide antibiotic resistance trend that has emerged not only within hospital settings, but also disseminated within the community. The limited available literature from African settings has reported the *mcr-1* gene mediated colistin resistance to be most prevalent in Africa, largely in South Africa, and this covers the largest portion of Africa according to the global map (Figure 1)\cite{4-7}. Beyond South Africa, the emergence of colistin resistance has been reported in Algeria, Rwanda and Uganda\cite{8-10}.

Evolution to plasmid mediated colistin resistance in gram-negative bacteria
Colistin (polymyxin E) is part of an old generation of antibiotics\cite{11} that form a family of cationic polypeptides. These are characterised by having a lipophilic fatty acyl side chain\cite{12-14}. No exact mechanism of bacterial killing has been documented for polymyxins, especially in *Acinetobacter* spp\cite{14,15}. However, a two-step mechanism has been described to elucidate their possible mechanism of action\cite{13,14}.

The two stages involve: i) initial binding to and permeabilization of the outer membrane and ii) the destabilisation of the cytoplasmic membrane of the bacteria\cite{12-14}. As a consequence, colistin functions by intercalating into the inner membrane following diffusion from the outer membrane across the periplasm and consequently causing the formation of pores, a phenomenon that results in bacterial lysis, which follows initial binding to bacterial surfaces\cite{12-14}. Initial binding of colistin to the bacterial surface chiefly depends on the electrostatic interaction between the positively-charged colistin and the negatively charged phosphate.
group of lipid A, an endo toxic component on the lipopolysaccharide localised on the outer leaflet of the bacterial outer membrane\textsuperscript{12,14}.

The modifications of the lipid A, which reduce and/or abolish the initial charge-based interaction with the polymyxins in bacteria\textsuperscript{13,15,16} and also the addition of either/or the 4-amino-4-deoxy-L-arabinose (L-Ara4N) and the phosphoethanolamine (PEtn) that ultimately form the basis of colistin resistance in bacteria\textsuperscript{16}, is mediated by chromosomally encoded genes. These are involved in the modulation of two component regulatory systems; PmrA/PmrB and PhoP/PhoQ and \textit{mcrB}, a negative regulator of the PhoP/PhoQ signalling system\textsuperscript{13-16}.

Although initially thought that this resistance could not be spread from cell to cell (plasmid mediated)\textsuperscript{16}, currently studies have shown otherwise. These have alluded transfer of colistin resistance among bacteria via plasmids in horizontal gene transfer\textsuperscript{14,16,17}. Plasmid transfer of the colistin resistance mobile genes, \textit{mcr-1}, \textit{mcr-2}, and also \textit{mcr-3}\textsuperscript{12-16}.

**Pan-drug resistance and characteristics of colistin resistant gram-negative bacteria**

The treatment of infections involving antibiotic resistant gram-negative bacteria has become increasingly difficult overtime, a factor that has greatly contributed to high morbidity, mortality and high costs of health care\textsuperscript{16,19}.

Currently, antibiotic resistance in these bacteria spans across several classes but likely follows a precise hierarchy of acquisition; this is mostly characterised by acquisition of “enhanced resistance” against more potent antibiotics following primary acquisition of “weaker resistance” against the less potent antibiotics alongside intrinsic resistance mechanisms in these bacteria, a trend that follows a Darwin’s like fashion\textsuperscript{20-23}. These changes are a function of horizontal gene transfer, via conjugation, transformation and transduction\textsuperscript{24-27}.

Resistance in gram-negative bacteria has been seen to transit from being mediated by the extended spectrum \textit{β}-lactamases, a group of enzymes that can be disseminated among bacteria\textsuperscript{18,20}; these chiefly confer resistance against broad spectrum cephalosporins. However, they also confer resistance to penicillins, monobactams and some carbapenems, particularly the \textit{Klebsiella pneumoniae} carbapenemase, KPC\textsuperscript{24-26,31}. In the same hierarchy are AmpC \textit{β}-lactamases that form another group of \textit{β}-lactamases, derived from older broad spectrum \textit{β}-lactamases. These provide an even more extended activity that includes resistance against the cephemycins alongside resistance to penicillins, monobactams and cephalosporins\textsuperscript{30-34}. These enzymes have in recent times been shown to not only be limited to being encoded on the chromosomes of bacteria, but have also been documented to have the potential of being disseminated via plasmids in horizontal gene transfer\textsuperscript{25,34,28} and also to co-exist with the extended spectrum \textit{β}-lactamases\textsuperscript{29,32}; factors that have made these bacteria “better resistant” to antibiotics. Next in the hierarchy are the carbapenemases, these enzymes are chiefly acquired in horizontal gene transfer and confer resistance to carbapenems alongside resistance to penicillins, broad spectrum cephalosporins including cefepime, a fourth generation cephalosporin, monobactams, aminoglycosides, quinolones and fluoroquinolones\textsuperscript{32,38}. The development of resistance mediated by these enzymes to the different classes of antibiotics in these bacteria has been attributed to various factors among which is their use in therapy. This has not only abetted maintenance of resistance via selecting for resistance to these antibiotics in these bacteria but has also created a gap, a need for alternative antibiotics in therapy to replace the penicillins, \textit{β}-lactams, carbapenems and the other classes of antibiotics used in the treatment of infections involving the drug resistant gram-negative bacteria\textsuperscript{4,16}.

Colistin, a polypeptide antibiotic, a relatively old antibiotic, has been currently relied upon to provide the ultimate line of refuge against infections caused by antibiotic resistant gram-negative bacteria despite its previously documented impacts on health\textsuperscript{4,16}. This provides a new challenge as bacteria that express these resistance genes assume the lead in the antibiotic resistance hierarchy and are distinctively extensive or worse pan drug resistant\textsuperscript{4,16-41}.

Molecular studies previously done have reported colistin resistant gram-negative bacteria to also be resistant to an array of antibiotics. These bacteria have also been reported to carry plasmids that have been found to carry alongside colistin resistance genes, \textit{β}-lactamases\textsuperscript{43,45}, carbapenemase encoding genes\textsuperscript{45} and genes that code for resistances to other antibiotic classes that may include quinolones, fluoroquinolones and aminoglycosides\textsuperscript{13}. Additionally, the carriage of \textit{mcr-1} has been documented as a possible indicator of resistance to the third generation cephalosporins and carbapenems\textsuperscript{38,44}. Furthermore, these genes have been found to be co-carried with other resistance determinants in plasmids\textsuperscript{34,44,46,47}; these genes represent a novel mechanism of antibiotic resistance in bacteria and a threat to the existing antibiotic therapy. Worsening the situation is the ability of selection for colistin resistance via the use of the extended spectrum cephalosporins. Additionally the use of tetracycline and sulphonamides has also been reported to contribute to the dissemination of colistin mobile gene carrying plasmids\textsuperscript{41,46}. Also, worth noting is plasmids that carry colistin resistance genes have also been found to mostly carry other antibiotic resistant genes\textsuperscript{13,44-46}.

**The role of high-throughput sequencing technologies and bioinformatics**

Advances in technology including the rapidly growing field of genomics, are transforming clinical medicine\textsuperscript{48} and high-throughput sequencing technology (HTS) is increasingly being used in clinical microbiology\textsuperscript{48}. HTS, with relatively simple bench top technology and efficient genomic library preparation protocols, has significantly improved the capacity to perform low-cost, efficient whole-genome sequencing (WGS), and has made it a feasible tool to enhance clinical diagnostic investigations in near
The processes generally involve culture-free parallel sequencing, producing vast quantities of genomic data that require modern computation techniques to assemble the genomic sequence reads as well as performing ensuing analyses that range from identifying the bacterial species or strain, antibiotic resistance mutations in the bacterial genomes, while ensuring the highest possible discriminatory power ever achieved by any technology. Apart from this, WGS of bacteria can identify genes associated with virulence and pathogenicity as well as discover new genetic mechanisms for virulence, pathogenicity and antibiotic resistance.

The identification and prediction of antibiotic resistant microorganisms in clinical specimens solely by molecular means in the diagnostic microbiology laboratory is not novel. HTS technologies and computational tools offer unprecedented ability to sequence multitudes of bacterial genomes and enable interpretation of the resultant sequence information in near “real-time”.

WGS represents the pinnacle for bacterial strain characterisation and epidemiological analyses. It is rapidly replacing traditional typing methods, antibiotic resistance gene detection and other molecular-based investigations in the near future. HTS technologies are rapidly evolving and their implementation in clinical and public health microbiology laboratories is increasing at a similar pace. These require standardised sample quality control, data interpretation, bioinformatics expertise, and infrastructure. The term ‘bioinformatics’ encompasses the handling and analysis of genomic sequence data, usually with the assistance of computer-based algorithms. Both ‘open source’ and commercially available bioinformatics programs/tools have been specifically developed for use in a clinical setting. However, many of practising healthcare workers in current practice have limited bioinformatics knowledge.

Furthermore, phenomena such as genome plasticity and pan genomes that have the ability to influence bacterial resistance can only effectively be investigated using HTS and bioinformatics analyses. Understanding the bacterial genome dynamics is an important step in identifying the forces behind the observed antibiotic resistance and therefore be able to effectively manage the disease in question.

The bottleneck that remains in implementing WGS for clinical purposes is post-sequencing data analysis.

Future direction of HTS
Antibiotic resistance in bacteria is generally a natural phenomenon though augmented by human behaviour. Therefore, it is imperative to harness the best HTS technologies that sequence DNA at unprecedented speed, to enable previously unimaginable scientific achievements and novel biological applications. Such applications of genomics tools has revolutionized microbial ecological studies and drastically expanded our view on the previously underappreciated microbial world including acquisition and transmission dynamics of antibiotic resistance. Single-Molecule Real-Time (SMRT) sequencing (Pacific BioSciences Inc.) in clinical microbiology has finally been realized at many levels in health care systems in the developing world and relatively only used during isolated scenarios of disease outbreaks in the less developed countries. These developments in HTS must be matched with continued efforts to improve the current bioinformatics analytic pipelines. Applying SMRT while genome sequencing to investigate bacterial colistin resistance would be made possible to predict resistance mutations, resistance mechanisms, trends, and patterns enabling efficient management of the colistin resistance by healthcare providers and pharmaceutical companies.

Conclusions
It is known that host, bacterial and environmental factors interact collectively to bring about antibiotic resistance. Therefore, HTS should be applied to a wide range of global collections of bacterial whole genomes to identify and predict new antibiotic drug resistance mutations using appropriate computational and bioinformatics algorithms.

Computational algorithms and tools offer ability to simulate bacterial genomic mutations while also offering possible clues on the mechanisms that may be shaping these mutations. These can as well be utilised to develop therapeutic interventions that may be used to target both the current and future acquired antibiotic drug resistance mutations.

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References


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