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T01

Processed foods from Bayelsa State Nigeria were potential reservoir for the occurrence and spread of multiple antibiotic resistance pathotypes of *Escherichia coli*

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The study aimed was to recover *E. coli* strains from processed ready-to-eat (RTE) foods in Yenagoa, Nigeria and characterised them using culture-based and molecular methods. Three hundred RTE food samples were collected randomly from different food outlets between February 2021 and August 2021 and assessed for the occurrence of *E. coli*, their virulence and antimicrobial resistance (AMR) characteristics using a standard bacteriological assay. The prevalence of *E. coli* was 80(26.7%). Sixty-two isolates of *E. coli* were confirmed using polymerase chain reaction (PCR) with specific primer sets and further characterised for antibiotic susceptibility and virulence properties. All the isolates were resistant to ≥2 antibiotics. The overall proportion of diarrheagenic *E. coli* was 33/62(53.2%). The distributions of typical diarrheagenic *E. coli* includes: tETEC 9(14.5%), tEPEC 13(20.9%), tEAEC 6(9.7%), tEIEC 2(3.2%) and tEHEC 3(4.8%). The proportions of atypical strains include aETEC 10(16.1%), aEAEC 5(8.1%), aEPEC 1(1.6%) and aEIEC 3(4.8%). This study demonstrated that some RTE foods sold in Yenagoa, Nigeria, are contaminated and constitute a probable human health hazard. This study thus emphasised the need for intensive surveillance of this isolate in RTE foods variety to spot evolving AMR phenotypes and avert food-borne infections.

T02

The rise of a megaplasmid family driving dissemination of multidrug resistance in Pseudomonas

Pseudomonas aeruginosa (Pa) is a WHO priority pathogen for which the role of plasmids in the emergence of multidrug resistance (MDR) has been systematically underestimated. Here we challenged this view by uncovering and characterising a family of megaplasmids (>370 kb) spreading MDR globally in the Pseudomonas genus. Using long-read sequencing we obtained complete sequences of related megaplasmids carrying large, complex, and dynamic arrays of resistance genes featuring extensive duplication and recombination events. Data mining, pangenome and phylogenomic analyses revealed that these IncP-2 plasmids identified in a hospital in Thailand are part of a multi-species family with broad ecological, geographical and temporal distribution. Many accessory genes in the family encode resistance determinants against diverse antibiotic classes, but also various other adaptive traits, highlighting their niche-dependent significance and a link between MDR observed in the clinic and environmental reservoirs. Despite their prominent size, we show evidence of high stability, low fitness cost, and efficient transmission in members of the family, underscoring the risk they pose to global health as effective MDR vehicles. In the short period since our first report (https://doi.org/10.1038/s41467-020-15081-7), the number of complete megaplasmid sequences has nearly quadrupled, opening the possibility to further our knowledge on their origin and evolution. A preliminary network analysis of their diversity revealed new distant relatives of different sizes and from various sources. We consider that this new approach, coupled with enhanced pangenome analyses, will unveil how megaplasmids are assembled and form the basis of their surveillance in both the clinic and the environment.

T03

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Implications of Plasmids and exogenous or cell-free DNA distribution on microbial resistome

Public health system has been saddened with the reoccurring reports as well as emergence of antimicrobial resistance especially as some of them have been source-tracked and associated with failure of antimicrobial therapy. Plasmids and cell-free DNA have been reported as culprit to such repeated occurrence both in clinical/environmental systems and removing them from the environment remains a way forward. Suffice to say that the sharing of such exogenous and/or cell-free nucleic acids (Plasmid DNA) occurs naturally in the environment (water, air, solid material and soil) without control by any known natural mechanism. It has been shown that such mechanism of sharing are associated with survival, stress and competition by such agents. Some of our previous studies have recommended that accumulated waste (liquid and solid) from hospital as well as environmental water sources and hygienic practitioners must ensure appropriate removal of such noxious agents to avert future epidemic/pandemic cases. The question of “at what level must such reduction be applied” became a major concern as it was suggested that practitioners must employ appropriately the water reuse policy and/or reduce exogenous DNA (plasmids) release with wastewater to a bearable minimum before release into the environment using specialized research-based methods (Nucleic Acid filteration). We believe that the removal of such exogenous DNA would impact horizontal resistance gene transfer (HRGT) and/or reduce acquisition of resistance genotype. This would also initiate appropriate steps to the control of plasmids distribution in the environment.

T04

Antibacterial Activity of *Terminalia superba* Engl. and Diels (Combretaceae) Barks Extracts Against Multidrug-Resistant Extended-Spectrum Beta-Lactamases (ESBLs) Producing Enterobacteriaceae Strains.

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Background: Improper use of antibiotics has led to a great concern in the development of pathogenic microbial resistance. The incidence of multidrug-resistance bacteria has been increasingly reported currently among Gram-positive. Extended-spectrum beta-lactamases (ESBLs) are an increasing cause of a multidrug-resistant in enterobacteriaceae, especially *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis.* The aim of the present study was to determine, *in vitro*, the activity of *T. superba* extracts against ESBL-producing enterobacteriaceae strains.
Methods: Total phenolic, total flavonoid, and total tannin contents of *T. superba* extracts were determined by spectrophotometric method. The strains were identified using API 20E tests. Antimicrobial susceptibility testing was performed by the disk diffusion test. ESBL-producing strains were detected using the double-disk test (amoxycillin clavulanic acid versus amoxicillin, imipenem, cefotaxime, ceftazidime, ceftriaxone, cefazolin, cefoxitin, aztreonam). ESBL production was further confirmed by hydrolysis of β-lactams of first, second and third generations. Antibacterial activity of *T. superba* extracts was estimated by the microdilution red phenol method. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the broth microdilution method using Mueller-Hinton broth and inocula of 10^6 CFU/ml with extracts concentrations from 80 to 0.078 mg/ml. Hydro-ethanolic extracts *of T. superba* were tested for their ability to inhibit the hydrolysis of nitrocefin by β-lactamase. After elimination of tannins (according to the European Pharmacopoeia) from the hydro-ethanolic extract, the extracts were also tested further for their anti-b-lactamase. The percentage inhibition of beta-lactamase was calculated and IC_{50} values were determined for each extract. Lower IC50 value shows higher radical scavenging activity.
Results: All tested strains (*Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis*) were resistant to amoxicillin, cefotaxime, ceftazidime, ceftriaxone, cefazolin, cefoxitin, aztreonam. The double-disk test was positive to detect ESBLs. Hydrolytic activity showed that all strains possessed ESBL. Values in different extracts showed that *T. superba* is the richest in polyphenols (total phenolic, total flavonoid, total tannin). The extracts induced inhibitory effects on beta-lactamase in a dose-dependent manner. The hydro-ethanolic extract exhibited potent inhibitory activity, respectively, of β-lactamases *ESBL-E. coli (IC_{50} = 0.065 mg/ml), ESBL-K.pneumoniae (IC_{50} = 0.076 mg/ml) and* ESBL-*P. mirabilis (IC_{50} =0.082 mg/ml)*. After elimination of tannins, anti-β-lactamase activity of hydro-ethanolic extract was weak. β-lactamases *ESBL-E. coli (IC_{50} = 0.134 mg/ml), ESBL-K. pneumoniae (IC_{50} = 0.166 mg/ml) and* ESBL-*P. mirabilis (IC_{50} =0.211 mg/ml)*.
Conclusion: *T. superba* extracts showed in vitro excellent activity against ESBL producing Enterobacteriaceae and may be clinically useful in treating infections caused by these pathogenics.

Keywords: *T. superba,* Antibacterial Activity, β-lactam, extended- spectrum beta-lactamases (ESBLs), β-lactamase inhibitors

T05

Characterization of Pipolins in Firmicutes shows a close evolutionary relationship between primer independent PolBs-coding elements and conjugative Mobile Genetic Elements.

Pipolins constitute a new class of Mobile Genetic Elements (MGEs) distinguished by encoding a primer-independent family B polymerase (piPolB). Found in all major bacterial clades and mitochondria, the study of pipolins in E. coli revealed that these elements are plastic att-flanked integrative MGEs present in a wide diversity of pathogenic strains. We carried out the first analysis of pipolins in Firmicutes in the GenBank database. Most of the pipolins detected belong to Staphylococcus, Limosilactobacillus, and other members of the Lactobacillaceae family. The lack of att-like direct repeats in more than 90% of pipolins suggests these elements may rely on unknown integration mechanisms or reside as circular plasmids. Previously known episomic pipolins such as pSE-12228-03 from Staphylococcus epidermidis and pLME300 from Limosilactobacillus fermentum supports the latter option. A sequence similarity network including our pipolins and plasmids in PLSDB shows that most staphylococcal pipolins are very similar to the plasmid TnSha2, which contains a copy of fabI associated with triclosan resistance. However, pipolins from Limosilactobacillus and other genera seem not to be related to any known plasmid. Pipolin gene clustering revealed that resolvases and relaxases are the most frequent encoded functions besides the piPolB, further supporting the idea that plasmids are the main form of pipolins in Firmicutes. However, the frequent presence of XerC-like integrases in pipolins from Lactobacillaceae indicates these elements might also be integrative. Finally, phylogenetic analysis of the piPolB, relaxases, and 16S rRNA genes showed incongruences that suggest the active horizontal transference of pipolins within the family boundaries.

T06

Detecting patterns of coevolution of gene gains and loss in bacterial species using data from thousands of bacterial genomes

Bacterial genomes exhibit widespread horizontal gene transfer, resulting in highly variable genome content that complicates the inference of genetic interactions. In this study, we develop a method for detecting coevolving genes from large datasets of bacterial genomes called the ``Close-Pair Score'' (CPS). The method is based on pairwise comparisons of closely related individuals, analogous to a pedigree study in eukaryotic populations. This approach avoids the need for an accurate phylogenetic tree and allows very large datasets to be analyzed, while focusing on recent coevolution. We apply our method to all of the more than 7 million pairs of genes from the entire annotated Staphylococcus aureus accessory genome of 2,756 annotated genes using a database of over 40,000 whole genomes. We find many pairs of genes that are frequently gained and lost together, as well as pairs where the gain of one gene is associated with the loss of the other. These pairs form networks of dozens of rapidly coevolving genes, primarily consisting of genes involved in metal resistance, virulence, mechanisms of horizontal gene transfer, and antibiotic resistance, particularly the SCCmec complex. Our results reflect the fact that the evolution of bacterial pathogens in the last half-century has largely been driven by antibiotic resistance gene gain, and in the case of S. aureus the SCCmec complex is the most prominent of these elements driving the evolution of resistance.

T07

An optimised short-read approach to predict and reconstruct antibiotic resistance plasmids in Escherichia coli

Escherichia coli has become the most prevalent resistant pathogen worldwide, being responsible for more than 250.000 deaths each year. Antibiotic resistance genes (ARGs) in E. coli are frequently encoded by plasmids, mobile genetic elements that play a pivotal role in the spread of resistance. Accurate reconstruction of E. coli ARG plasmids from Illumina sequencing data has proven to be a challenge with current bioinformatic tools. In this work, we present an improved method to reconstruct E. coli plasmids using short reads. We developed an ensemble classifier, named plasmidEC, that identifies plasmid-derived contigs by combining the output of three binary classification tools. We showed that plasmidEC is especially suited to classify contigs derived from ARG plasmids with a recall of 0.941. Additionally, we optimised gplas, a graph-based tool that bins plasmid-predicted contigs into distinct plasmid predictions. This new version of gplas is more effective at recovering plasmids with large sequencing coverage variations and can be combined with the output of any binary classification tool. The combination of plasmidEC with gplas showed a high recall (median=0.818) and F1-score (median=0.812) when reconstructing ARG plasmids, and exceeded the binning capacity of the reference-based method MOB-suite. In the absence of long read data, our method offers the best alternative to reconstruct ARG plasmids in E. coli.

T08

Antibiotic resistance on multicopy plasmids

Plasmids often exist in multiple copies within a single bacterial cell. This has consequences for the evolution of antibiotic resistance on plasmids. Multicopy plasmids increase the mutational target size for resistance mutations and allow for gene dosage effects. A crucial feature of multicopy plasmids is that the segregation of plasmid replicates into the daughter cells leads to bacteria with a plasmid composition different from that of the mother cell, i.e., the number of mutated plasmid copies may differ between mother and daughter cells. Here, we present our findings on the influence of the plasmid copy number on the probability of resistance evolution on multicopy plasmids. For this, we developed a mathematical model where successful adaptation relies on the establishment of de novo mutations on plasmids. Our results show that chances of resistance evolution increase or decrease with the plasmid copy number depending on the dominance relationship between mutant and wild-type alleles. Using a simple model of antibiotic degradation within bacterial cells, we show that the dominance function may change with the antibiotic concentration. Moreover, we consider the fixation process of dominant mutant alleles on a multicopy plasmid. We find that the beneficial mutant phenotype may fix in the population faster than the mutant homozygous genotype. We term this time interval the heterozygosity window and show that it emerges if the copy number is high and selection strong. The length of this ‘heterozygosity window’ has consequences for the reversibility of resistance when antibiotics are removed.

T09

Klebsiella genomic epidemiology revealed potential hospital transmission and the presence of hybrid resistance and virulence plasmid in Qatar

The molecular epidemiology of carbapenem-resistant Klebsiella species is not well investigated in Qatar. This investigation aimed to characterize the genetic context of carbapenemase-producing Klebsiella strains recovered from clinical specimens using whole genome sequencing (WGS) approach. Klebsiella isolates (n=100) were collected at 7 tertiary hospitals from 2015-2017. Phylogenomic analysis, screening of resistance and virulence genes, and comparison of genetic environment of carbapenemase were carried out after WGS. Our data indicated that K. pneumoniae was common (80), followed by K. quasipneumoniae (16), K. aerogenes (3) and K. oxytoca (1). The most prevalent were genes encoding NDM-1 (39), OXA-48 (20), OXA-232 (10) and OXA-181 (12). KPC-2 (3) and KPC-3 (2) were also identified. Plasmid locations of 24 carbapenemase-encoding genes were determined; blaNDM-1 was localized on IncFII replicon, while blaOXA-181 and blaOXA-232 were commonly associated with ColKP3 plasmids. pOXA-48-like plasmid was detected in 17/20 isolates harboring blaOXA-48. MLSTs were diverse and the 'traditional' clonal group (CG) 258 was rare. K. pneumoniae ST147 was predominant (13), followed by ST231 (7) and ST11 (5). Nine K. quasipneumoniae isolates belonged to ST196 and were highly clonal. The virulence loci such as yersiniabactin (ybt) and rmpA were not detected within the study's K. quasipneumoniae isolates. Amongst K. pneumoniae, there were 50 ybt+ isolates; 8 isolates had rmpA, and of these, 3 belonged to ST383. One of CR-hv isolates (ST383) had plasmids that carried both carbapenemase genes and multiple virulence factors. The detection of carbapenemase-producing isolate bearing rmpA and NDMs reflect the presence of multidrug resistance and virulence strain.