Direct whole-genome sequencing of Plasmodium falciparum specimens from dried erythrocyte spots

Background: Plasmodium falciparum malaria remains a major health burden and genomic research represents one of the necessary approaches for continued progress towards malaria control and elimination. Sample acquisition for this purpose is troublesome, with the majority of malaria-infected individuals living in rural areas, away from main infrastructure and the electrical grid. The aim of this study was to describe a low-tech procedure to sample P. falciparum specimens for direct whole genome sequencing (WGS), without use of electricity and cold-chain.

Methods: Venous blood samples were collected from malaria patients in Bandim, Guinea-Bissau and leukocyte-depleted using Plasmodipur filters, the enriched parasite sample was spotted on Whatman paper and dried. The samples were stored at ambient temperatures and subsequently used for DNA-extraction. Ratios of parasite:human content of the extracted DNA was assessed by qPCR, and five samples with varying parasitaemia, were sequenced. Sequencing data were used to analyse the sample content, as well as sample coverage and depth as compared to the 3d7 reference genome. Results: qPCR revealed that 73% of the 199 samples were applicable for WGS, as defined by a minimum ratio of parasite:human DNA of 2:1. WGS revealed an even distribution of sequence data across the 3d7 reference genome, regardless of parasitaemia. The acquired read depths varied from 16 to 99×, and coverage varied from 87.5 to 98.9% of the 3d7 reference genome. SNP-analysis of six genes, for which amplicon sequencing has been performed previously, confirmed the reliability of the WGS-data.

Conclusion: This study describes a simple filter paper based protocol for sampling P. falciparum from malaria patients for subsequent direct WGS, enabling acquisition of samples in remote settings with no access to electricity.
Selective enrichment of ESBL, AmpC and carbapenemase producing E. coli in meat and cecal samples - additional validation for poultry samples

General information
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Organisations: National Food Institute, Research Group for Genomic Epidemiology
Authors: Cavaco, L. (Intern), Hendriksen, R. S. (Intern), Agerse, Y. (Intern), Svendsen, C. A. (Intern), Nielsen, H. (Ekstern), Guerra, B. (Ekstern), Peran, R. (Ekstern), Hasman, H. (Ekstern)
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Genomic Signature of Multidrug-Resistant Salmonella enterica Serovar Typhi Isolates Related to a Massive Outbreak in Zambia between 2010 and 2012.

Retrospectively, we investigated the epidemiology of a massive Salmonella enterica serovar Typhi outbreak in Zambia during 2010 to 2012. Ninety-four isolates were susceptibility tested by MIC determinations. Whole-genome sequence typing (WGST) of 33 isolates and bioinformatic analysis identified the multilocus sequence type (MLST), haplotype, plasmid replicon, antimicrobial resistance genes, and genetic relatedness by single nucleotide polymorphism (SNP) analysis and genomic deletions. The outbreak affected 2,040 patients, with a fatality rate of 0.5%. Most (83.0%) isolates were multidrug resistant (MDR). The isolates belonged to MLST ST1 and a new variant of the haplotype, H58B. Most isolates contained a chromosomally translocated region containing seven antimicrobial resistance genes, catA1, blaTEM-1, dfrA7, sul1, sul2, strA, and strB, and fragments of the incompatibility group Q1 (IncQ1) plasmid replicon, the class 1 integron, and the mer operon. The genomic analysis revealed 415 SNP differences overall and 35 deletions among 33 of the isolates subjected to whole-genome sequencing. In comparison with other genomes of H58, the Zambian isolates separated from genomes from Central Africa and India by 34 and 52 SNPs, respectively. The phylogenetic analysis indicates that 32 of the 33 isolates sequenced belonged to a tight clonal group distinct from other H58 genomes included in the study. The small numbers of SNPs identified within this group are consistent with the short-term transmission that can be expected over a period of 2 years. The phylogenetic analysis and deletions suggest that a single MDR clone was responsible for the outbreak, during which occasional other S. Typhi lineages, including sensitive ones, continued to cocirculate. The common view is that the emerging global S. Typhi haplotype, H58B, containing the MDR IncHI1 plasmid is responsible for the majority of typhoid infections in Asia and sub-Saharan Africa; we found that a new variant of the haplotype harboring a chromosomally translocated region containing seven antimicrobial resistance genes, catA1, blaTEM-1, dfrA7, sul1, sul2, strA, and strB, and fragments of the incompatibility group Q1 (IncQ1) plasmid replicon, the class 1 integron, and the mer operon. The genomic analysis revealed 415 SNP differences overall and 35 deletions among 33 of the isolates subjected to whole-genome sequencing. In comparison with other genomes of H58, the Zambian isolates separated from genomes from Central Africa and India by 34 and 52 SNPs, respectively. The phylogenetic analysis indicates that 32 of the 33 isolates sequenced belonged to a tight clonal group distinct from other H58 genomes included in the study. The small numbers of SNPs identified within this group are consistent with the short-term transmission that can be expected over a period of 2 years. The phylogenetic analysis and deletions suggest that a single MDR clone was responsible for the outbreak, during which occasional other S. Typhi lineages, including sensitive ones, continued to cocirculate. The common view is that the emerging global S. Typhi haplotype, H58B, containing the MDR IncHI1 plasmid is responsible for the majority of typhoid infections in Asia and sub-Saharan Africa; we found that a new variant of the haplotype harboring a chromosomally translocated region containing the MDR islands of IncHI1 plasmid has emerged in Zambia. This could change the perception of the term "classical MDR typhoid" currently being solely associated with the IncHI1 plasmid. It might be more common than presently thought that S. Typhi haplotype H58B harbors the IncHI1 plasmid or a chromosomally translocated MDR region or both.

General information
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Human populations worldwide are increasingly confronted with infectious diseases and antimicrobial resistance spreading faster and appearing more frequently. Knowledge regarding their occurrence and worldwide transmission is important to control outbreaks and prevent epidemics. Here, we performed shotgun sequencing of toilet waste from 18 international airplanes arriving in Copenhagen, Denmark, from nine cities in three world regions. An average of 18.6 Gb (14.8 to 25.7 Gb) of raw Illumina paired end sequence data was generated, cleaned, trimmed and mapped against reference sequence databases for bacteria and antimicrobial resistance genes. An average of 106,839 (0.06%) reads were assigned to resistance genes with genes encoding resistance to tetracycline, macrolide and beta-lactam resistance genes as the most abundant in all samples. We found significantly higher abundance and diversity of genes encoding antimicrobial resistance, including critical important resistance (e.g. bla\textsubscript{CTX-M}) carried on airplanes from South Asia compared to North America. Presence of \textit{Salmonella enterica} and norovirus were also detected in higher amounts from South Asia, whereas \textit{Clostridium difficile} was most abundant in samples from North America. Our study provides a first step towards a potential novel strategy for global surveillance enabling simultaneous detection of multiple human health threatening genetic elements, infectious agents and resistance genes.

**General information**

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Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, Metagenomics, National Food Institute, Research Group for Genomic Epidemiology, Research Group for Diagnostic Engineering  
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Emergence and clonal dissemination of Salmonella enterica serovar Enteritidis causing salmonellosis in Mauritius

Introduction: For decades, Salmonella enterica serovar Enteritidis has been among the most prevalent serovars reported worldwide. However, it was rarely encountered in Mauritius until 2007; since then the number of non-typhoidal Salmonella serogroup O:9 (including serovar Enteritidis) increased. A study was conducted to investigate the genetic relatedness between S. Enteritidis isolates recovered in Mauritius from food and clinical specimens (stool, blood, and exudate).

Methodology: Forty-seven isolates of S. Enteritidis obtained in 2009 from human stools, blood cultures and exudates, and from food specimens were characterized by antimicrobial susceptibility testing and Multiple-Locus Variable-number tandem repeat Analysis (MLVA).

Results: With the exception of a single isolate which demonstrated intermediate susceptibility to streptomycin, all isolates were pansusceptible to the 14 antimicrobials tested. Thirty seven out of the 47 isolates (78.7%) exhibited an indistinguishable MLVA profile which included isolates from ready-to-eat food products, chicken, and human clinical isolates from stool, blood and exudate.

Conclusions: The presence of highly related strains in both humans and raw chicken, and the failure to isolate the serovar from other foods, suggests that poultry is the main reservoir of S. Enteritidis in Mauritius and that the majority of human cases are associated with chicken consumption which originated from one major producer. Stool isolates were indistinguishable or closely related to blood and exudate isolates, indicating that, besides gastroenteritis, the same strain caused invasive infections. Control of S. Enteritidis by poultry breeders would lower the financial burden associated with morbidity in humans caused by this organism in Mauritius.
We report the genetic characterization of 15 Klebsiella pneumoniae (KP) and 4 isolates of K. oxytoca (KO) from clinical cases in dogs and cats and showing extended-spectrum cephalosporin (ESC) resistance. Extended spectrum beta-lactamase (ESBL) and AmpC genes, plasmid-mediated quinolone resistance (PMQR) and co-resistances were investigated. Among KP isolates, ST101 clone was predominant (8/15, 53%), followed by ST15 (4/15, 27%). ST11 and ST340, belonging to Clonal Complex (CC) 11, were detected in 2012 (3/15, 20%). MLST on KP isolates corresponded well with PFGE results, with 11 different PFGE patterns observed, including two clusters of two (ST340) and four (ST101) indistinguishable isolates, respectively. All isolates harbored at least one ESBL or AmpC gene, all carried on transferable plasmids (IncR, IncFII, IncI1, IncN), and 16/19 were positive for PMQR genes (qnr family or aac(6')-Ib-cr). The most frequent ESBL was CTX-M-15 (11/19, 58%), detected in all KP ST101, in one KP ST15 and in both KP ST340. blaCTX-M-15 was carried on IncR plasmids in all but one KP isolate. All KP ST15 isolates harbored different ESC resistance genes and different plasmids, and presented the non-transferable bla(SHV-28) gene, in association with blaCTX-M-15, blaCTX-M-1 (on IncR, or on IncN), bla(SHV-2a) (on IncR) or bla(CMY-2) genes (on IncI1), KO isolates were positive for blaCTX-M-9 gene (on IncHI2), or for the bla(SHV-12) and bla(DHA-1) genes (on IncL/M). They were all positive for qnr genes, and one also for the aac(6')-Ib-cr gene. All Klebsiella isolates showed multiresistance towards aminoglycosides, sulfonamides, tetracyclines, trimethoprim and amphenicols, mediated by strA/B, aadA2, aadB, ant(2")-Ia, aac(6')-Ib, sul, tet, dfr and cat genes in various combinations. The emergence in pets of multidrug-resistant Klebsiella with ESBL, AmpC and PMQR determinants, poses further and serious challenges in companion animal therapy and raise concerns for possible bidirectional transmission between pets and humans, especially at household level.
MULTIDISCIPLINARY, FRAGMENT LENGTH POLYMORPHISM, ESCHERICHIA-COLI, MULTIPLEX PCR, DETERMINANTS QNR, PNEUMONIAE CLONE, GENES, PREVALENCE, ENTEROBACTERIACEAE, AAC(6')-IB-CR, OUTBREAK
Rapid whole genome sequencing for the detection and characterization of microorganisms directly from clinical samples.

Whole genome sequencing (WGS) is becoming available as a routine tool for clinical microbiology. If applied directly on clinical samples this could further reduce diagnostic time and thereby improve control and treatment. A major bottleneck is the availability of fast and reliable bioinformatics tools. This study was conducted to evaluate the applicability of WGS directly on clinical samples and to develop easy-to-use bioinformatics tools for analysis of the sequencing data. Thirty-five random urine samples from patients with suspected urinary tract infections were examined using conventional microbiology, WGS of isolated bacteria and by directly sequencing on pellets from the urine. A rapid method for analyzing the sequence data was developed. Bacteria were cultivated from 19 samples, but only in pure culture from 17. WGS improved the identification of the cultivated bacteria and almost complete agreement was observed between phenotypic and predicted antimicrobial susceptibility. Complete agreement was observed between species identification, multi-locus-sequence typing and phylogenetic relationship for the Escherichia coli and Enterococcus faecalis isolates when comparing the results of WGS of cultured isolates and directly from the urine samples. Sequencing directly from the urine enabled bacterial identification in polymicrobial samples. Additional putative pathogenic strains were observed in some culture negative samples. WGS directly on clinical samples can provide clinically relevant information and drastically reduce diagnostic time. This may prove very useful, but the need for data analysis is still a hurdle to clinical implementation. To overcome this problem a publicly available bioinformatics tool was developed in this study.

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Dissemination of clonal Salmonella enterica serovar Typhimurium isolates causing salmonellosis in Mauritius

Salmonella enterica serotype Typhimurium is one of the leading causes of salmonellosis in Mauritius, where it has also been associated with outbreaks of foodborne illness. However, little is known about its molecular epidemiology in the country. This study was therefore undertaken to investigate the clonality and source of Salmonella Typhimurium in Mauritius by studying human, food, and poultry isolates by pulsed-field gel electrophoresis (PFGE) and antibiotic minimum inhibitory concentration determination. Forty-nine isolates collected between 2008 and 2011 were analyzed, including 25 stool isolates from foodborne illness outbreaks and sporadic gastroenteritis cases, four blood isolates, one postmortem colon isolate, 14 food isolates, and five poultry isolates. All isolates were pan-susceptible to the 16 antibiotics tested, except for two isolates that were resistant to sulfamethoxazole and trimethoprim. Overall characterization of the isolates by PFGE digested with XbaI and BlnI resulted in eight different patterns. The largest of the clusters in the composite dataset consisted of 20 isolates, including two raw chicken isolates, four poultry isolates, and nine human stool isolates from two outbreaks. A second cluster consisted of 18 isolates, of which 12 originated from human blood and stool samples from both sporadic and outbreak cases. Six food isolates were also found in this cluster, including isolates from raw and grilled chicken, marlin mousse, and cooked pork. One poultry isolate had a closely related PFGE pattern. The results indicate that one clone of Salmonella Typhimurium found in poultry has been causing outbreaks of foodborne illness in Mauritius and another clone that has caused many cases of gastrointestinal illness and bacteremia in humans could also be linked to poultry. Thus, poultry appears to be a major reservoir for Salmonella Typhimurium in Mauritius. Initiating on-farm control strategies and measures against future dissemination may substantially reduce the number of cases of salmonellosis in the country.

General Information
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Scopus rating (2013): SJR 1.167 SNIP 1.15 CiteScore 2.41
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Genomics of an emerging clone of Salmonella serovar Typhimurium ST313 from Nigeria and the Democratic Republic of Congo.
We showed in a limited number of isolates that S. Typhimurium ST313 is a prevalent sequence-type causing gastrointestinal diseases and septicemia in patients from Nigeria and DRC. We found three distinct phylogenetic clusters based on the origin of isolation suggesting some spatial evolution. Comparative genomics showed an interesting putative virulence fragment (ST313-TD) unique to S. Typhimurium ST313 and invasive S. Dublin.

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Organisations: Comparative Microbial Genomics, National Food Institute, Division of Epidemiology and Microbial Genomics, Center for Biological Sequence Analysis, Immunological Bioinformatics, Division of Microbiology and Risk Assessment, Translational Genomics Research Institute, University of Ibadan, Saint-Pierre University Hospital
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Molecular clonality and antimicrobial resistance in Salmonella enterica serovars Enteritidis and Infantis from broilers in three Northern regions of Iran

ABSTRACT: BACKGROUND: Multidrug-resistant Salmonella strains are frequently encountered problems worldwide with considerable increased occurrences in recent years. The aim of this study was to investigate the occurrence and frequency of antimicrobial resistance and associated resistance genes in Salmonella isolates from broiler farms in different regions of Iran covering a time period of four years. RESULTS: From 2007 to 2011, 36 Salmonella strains were isolated from broiler farms located in three northern provinces of Iran. The isolates were serotyped, antimicrobial susceptibility tested, and characterized for antimicrobial resistance genes associated to the phenotype. Pulsed-field gel electrophoresis (PFGE) was applied for comparison of genetic relatedness. Two serovars were detected among the isolates; Salmonella enterica serovar Infantis (75%) and S. Enteritidis (25%). Thirty-four (94%) of the isolates exhibited resistance to nalidixic acid and ciprofloxacin caused by a single mutation in the quinolone resistance-determining region (QRDR) of gyrA. For all strains this mutation occurred in the codon of Asp87 leading to Asp87-Tyr, Asp87-Gly or Asp87-Asn substitutions. All S. Infantis (n = 27) were resistant to tetracycline, spectinomycin, streptomycin, and sulfamethoxazole and harbored the associated resistance genes; tetA, dfrA14, aadA1, and sul1 together with class 1 integrons. The isolates revealed highly similar PFGE patterns indicating clonal relatedness across different geographical locations. CONCLUSION: The data provided fundamental information applicable when launching future control programs for broilers in Iran with the aim to conserve the effectiveness of important antimicrobials for treatment in humans.

General information
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Organisations: National Food Institute, Division of Epidemiology and Microbial Genomics, University of Tehran
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Occurrence of Listeria spp. in retail meat and dairy products in the area of Addis Ababa, Ethiopia.

Background. Listeriosis, a bacterial disease in humans and animals, is mostly caused by ingestion of Listeria monocytogenes via contaminated food and/or water, or by a zoonotic infection. Globally, listeriosis has in general a low incidence but a high case fatality rate. Objective. The objective of this study was to investigate the occurrence, antimicrobial profiles, and genetic relatedness of L. monocytogenes from raw meat and dairy products (raw milk, cottage cheese, cream cake), collected from the capital and five neighboring towns in Ethiopia. Methods. Two hundred forty food samples were purchased from July to December 2006 from food vendors, shops, and supermarkets, using a cross-sectional study design. L. monocytogenes were isolated and subjected to molecular serotyping. The genetic relatedness and antimicrobial susceptibility patterns were investigated using pulsed-field gel electrophoresis (PFGE) and minimum inhibitory concentration determinations. Results. Of 240 food samples tested, 66 (27.5%) were positive for Listeria species. Of 59 viable isolates, 10 (4.1%) were L. monocytogenes. Nine were serotype 4b and one was 2b. Minimum inhibitory concentration determination and PFGE of the 10 L. monocytogenes isolates showed low occurrence of antimicrobial resistance among eight different PFGE types. Discussion and Conclusions. The findings in this study correspond to similar research undertaken in Ethiopia by detecting L. monocytogenes with similar prevalence rates. Public education is crucial as regards the nature of this organism and relevant prevention measures. Moreover, further research in clinical samples should be carried out to estimate the prevalence and carrier rate in humans, and future investigations on foodborne outbreaks must include L. monocytogenes.

General information
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Organisations: National Food Institute, Division of Epidemiology and Microbial Genomics, Ethiopian Health and Nutrition Research Institute, Addis Ababa University, Agence nationale de la sécurité sanitaire, alimentation, environnement et travail
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The first attempt of an active integrated laboratory-based Salmonella surveillance programme in the north-eastern region of Nigeria

To identify the sources of Salmonella contamination, distribution, prevalence and antimicrobial susceptibility patterns, which have significant impact on public and animal health, and international trade. A total of 1888 samples were collected
by stratified random sampling from 2009 to 2011 from cattle, camels, poultry, fish, vegetables and humans. All identified Salmonella isolates were serotyped and tested for antimicrobial susceptibility by MIC determinations. A total of 149 Salmonella isolates comprising 17 different serovars were obtained (7.9% prevalence). Salmonella Hadar (37%), S. Eko (17%), S. Enteritidis (10%), S. Kentucky (7%) and S. Uganda (7%) were isolated from different sources. The occurrence of antimicrobial resistance was generally low, but S. Enteritidis and S. Eko showed variable antimicrobial resistance patterns, while all S. Kentucky isolates were resistant to seven of 17 tested antimicrobials, including ciprofloxacin and nalidixic acid. Three S. Hadar isolates revealed reduced susceptibility to ciprofloxacin and susceptibility to nalidixic acid and harboured the plasmid-mediated quinolone resistance gene qnrS1. Salmonella serovars Hadar, Enteritidis and the previously very rarely reported Eko were the major serovars associated with human infections, animal and environmental contamination in the north-eastern region of Nigeria. These serovars constitute a health risk to poultry, environment and human population in the region.

General information
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Organisations: National Food Institute, Division of Epidemiology and Microbial Genomics, University of Ilorin, University of Maiduguri
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  Scopus rating (2014): CiteScore 2.56
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  BFI (2013): BFI-level 1
  Scopus rating (2013): CiteScore 2.69
  ISI indexed (2013): ISI indexed yes
  Web of Science (2013): Indexed yes
  BFI (2012): BFI-level 1
  Scopus rating (2012): CiteScore 2.51
  ISI indexed (2012): ISI indexed yes
  Web of Science (2012): Indexed yes
  BFI (2011): BFI-level 1
  Scopus rating (2011): CiteScore 2.55
  ISI indexed (2011): ISI indexed yes
  Web of Science (2011): Indexed yes
  BFI (2010): BFI-level 1
  Web of Science (2010): Indexed yes
  BFI (2009): BFI-level 1
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  BFI (2008): BFI-level 1
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Antimicrobial-resistant Shigella infections from Iran: an overlooked problem?

Objectives: In this study, we wanted to assess the level of antimicrobial resistance, the presence of genes encoding resistance to cephalosporins and plasmid-mediated quinolone resistance (PMQR), and genetic relatedness among Shigella isolates obtained from Iranian patients. Methods: A total of 44 Shigella isolates were collected from Iranian patients admitted to Milad Hospital, Tehran, Iran, during 2008–10. Of these, 37 were serotyped and characterized by MIC determination. A subset of eight suspected extended-spectrum β-lactamase (ESBL) producers (six Shigella sonnei phase II and two Shigella flexneri type 1b) were examined for the presence of genes encoding cephalosporin resistance. The presence of PMQR was assessed in one S. flexneri isolate exhibiting low-level resistance to ciprofloxacin and susceptibility to nalidixic acid. PFGE was performed on 25 S. sonnei phase II isolates. Results: Of the isolates, 25 (68%) were S. sonnei phase II, with 5 (14%) S. flexneri, 5 (14%) Shigella dysenteriae type 2, and 2 (5%) Shigella boydii type 2. Resistance to at least three classes of antimicrobials was detected in all species. The presence of blaCTX-M-15 and the AmpC β-lactamase producer blaCMY-2 was confirmed in five and one S. sonnei phase II isolates, respectively. One of the two S. flexneri type 1b that contained blaCTX-M-15 also harboured a qnrS1 gene. PFGE identified seven PFGE profiles; the main cluster included 15 of the strains, suggesting low genetic diversity between isolates or the presence of an endemic clone in Iran. Conclusions: This is the first known description of ESBL-producing and AmpC β-lactamase-producing Shigella and of PMQR Shigella in Iran. The emergence of CTX-15, CMY-2 and qnrS1 genes may compromise the treatment of shigellosis. Strategies to minimize the spread of ESBL-producing and AmpC β-lactamase-producing Shigella should be implemented.
Characterization of Isolates of Salmonella enterica Serovar Stanley, a Serovar Endemic to Asia and Associated with Travel

Salmonella enterica serovar Stanley (S. Stanley) is a common serovar in Southeast Asia and was the second most common serovar implicated in human salmonellosis in Thailand in the years 2002 to 2007. In contrast, this serovar is relatively uncommon in Europe. The objective of this study was to characterize a collection of S. Stanley strains isolated from Thai (n = 62), Danish (n = 39), and French (n = 24) patients to gain a broader understanding of the genetic diversity, population dynamics, and susceptibility to antimicrobials. All isolates were characterized by pulsed-field gel electrophoresis and antimicrobial susceptibility testing. The molecular mechanisms of resistance to extended-spectrum cephalosporins and plasmid-mediated resistance to quinolones were characterized by PCR and sequencing. Plasmid profiling, replicon typing, and microarray analysis were used to characterize the genetic mechanisms of antimicrobial resistance in 10 extended-spectrum cephalosporinase-producing isolates. Considerable genetic diversity was observed...
among the isolates characterized with 91 unique XbaI pulsed-field gel electrophoresis (PFGE) patterns, including 17 distinct clusters consisting of two to seven indistinguishable isolates. We found some of the S. Stanley isolates isolated from patients in Europe were acquired during travel to Southeast Asia, including Thailand. The presence of multiple plasmid lineages carrying the extended-spectrum cephalosporinase-encoding \( \text{bla}_{\text{CMY-2}} \) gene in S. Stanley isolates from the central part of Thailand was confirmed. Our results emphasize that Thai authorities, as well as authorities in other countries lacking prudent use of antimicrobials, should improve the ongoing efforts to regulate antimicrobial use in agriculture and in clinical settings to limit the spread of multidrug-resistant *Salmonella* isolates and plasmids among humans and pigs in Thailand and abroad.

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Characterization of Salmonella enterica serovar Enteritidis isolates recovered from blood and stool specimens in Thailand

The increased percentage of bloodstream infections as described in the 2009 observational study could not be attributed to a single clone. Future efforts should focus on assessing the immune status of bacteriaemic patients and identifying prevention and control measures, including attribution studies characterizing non-clinical (animal, food, and environmental) isolates.

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SCCmec Type IX Element in Methicillin Resistant Staphylococcus aureus Sp type t337 (CC9) Isolated from Pigs and Pork in Thailand

Methicillin resistant Staphylococcus aureus (MRSA) have emerged among livestock in several countries. In this study, we describe the results of a screening performed in pigs and raw pork samples in Thailand. Ten pork samples and 15 nasal swabs from pigs were collected from 2 markets and 1 pig farm in the Samuth Songkhram province in Thailand. MRSA were isolated using selective isolation procedures and confirmed by mecA PCR. The MRSA were characterized by antimicrobial susceptibility testing, pulsed field gel electrophoresis (PFGE), spa typing, SCCmec typing, and MLST. Resistance and virulence markers were screened using a microarray. Five of the pork samples and six pig nasal swabs were positive for MRSA. All 11 isolates belonged to spa type t337 but showed diversity in antimicrobial resistance patterns and PFGE profiles. Additionally, the isolates were sequence-typed; ST9, ST2136, ST2278 belonging to the clonal complex; CC9. All isolates harbored SCCmec IX and were resistant to 7 out of 14 tested antimicrobials; additional resistances to all antimicrobials tested were found in some of the pork and pig isolates and 1 pork isolate was resistant to 13 antimicrobials tested. Microarray analysis identified blaZ, aac-aphD, vga(A), tetM, and a tet efflux marker, in all strains and additionally ermB and aadD, cat and fex(A) in the pork isolates. None of the isolates were found PVL-positive, but
enterotoxins were identified in all isolates. To our knowledge, only a few descriptions of MRSA in livestock and food products in Thailand have been observed but this is the first observation of MRSA CC9 associated with SCCmec IX in pork. This study indicates a likely widespread distribution of MRSA in pig and pork in Thailand and further investigation on the prevalence and importance of livestock associated MRSA in Thailand is needed.

Relevance of hot spots in the evolution and transmission of Tn1546 in glycopeptide-resistant Enterococcus faecium (GREF) from broiler origin

Objectives: Glycopeptide-resistant enterococci are still present within the broiler sector, despite the EU ban of avoparcin more than a decade ago. In the present study, we have developed a rapid method for screening the flanking regions at the integration point of Tn1546 in glycopeptide-resistant Enterococcus faecium isolated from broiler farms. Methods: Total DNA was digested, ligated and amplified using primers from inside Tn1546. The resulting amplicons were purified and sequenced. Two new primers were designed based on obtained sequences. Results: Two main insertion points have been repeatedly found in isolates from the UK (n = 150). The first insertion point revealed that 25 isolates harboured Tn1546 positioned in a sequence with 96% homology to a streptomycin adenyltransferase gene (AY604739) from a
Staphylococcus intermedius plasmid. At this insertion point, a direct repeat (GTCCT) was duplicated as previously described, indicating transposition at the target site. Furthermore, this 'hot spot' was also detected in isolates from Norway (2/8) and Denmark (17/20). The second insertion point detected in 45 isolates from the UK revealed integration into an Inc18-like plasmid, most likely by a process of target site recombination. Conclusions: The presence of a common insertion point for isolates from different geographical areas could suggest the insertion of Tn1546 by transposition in a plasmid-specific site, followed by genetic rearrangement both inside the transposon and in the flanking regions.

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