Infect Immun. 2012 May;80(5):1815-22. doi: 10.1128/IAI.06395-11. Epub 2012 Feb 21.

Loss of outer membrane protein C in Escherichia coli contributes to both antibiotic resistance and escaping antibody-dependent bactericidal activity.

Liu YF, Yan JJ, Lei HY, Teng CH, Wang MC, Tseng CC, Wu JJ.

Source

Institutes of Basic Medical Sciences, National Cheng-Kung University, Tainan, Taiwan.

Abstract

Outer membrane proteins (OMPs) serve as the permeability channels for nutrients, toxins, and antibiotics. In Escherichia coli, OmpA has been shown to be involved in bacterial virulence, and OmpC is related to multidrug resistance. However, it is unclear whether OmpC also has a role in the virulence of E. coli. The aims of this study were to characterize the role of OmpC in antimicrobial resistance and bacterial virulence in E. coli. The ompC deletion mutant showed significantly decreased susceptibility to carbapenems and cefepime. To investigate the survival of E. coli exposed to the innate immune system, a human blood bactericidal assay showed that the ompC mutant increased survival in blood and serum but not in complement-inactivated serum. These effects were also demonstrated in the natural selection of OmpC mutants. Also, C1q interacted with E. coli through a complex of antibodies bound to OmpC as a major target. Bacterial survival was increased in the wild-type strain in a dose-dependent manner by adding free recombinant OmpC protein or anti-C1q antibody to human serum. These results demonstrated that the interaction of OmpC-specific antibody and C1q was the key step in initiating the antibody-dependent classical pathway for the clearance of OmpC-expressing E. coli. Anti-OmpC antibody was detected in human sera, indicating that OmpC is an immunogen. These data indicate that the loss of OmpC in E. coli is resistant to not only antibiotics, but also the serum bactericidal effect, which is mediated from the C1q and anti-OmpC antibody-dependent classical pathway.

Supplemental Content

BMC Genomics. 2011 Nov 28;12:583.

The association of DNA damage response and nucleotide level modulation with the

antibacterial mechanism of the anti-folate drug trimethoprim.

Sangurdekar DP, Zhang Z, Khodursky AB.

Source

Lewis-Sigler Institute for Integrative Genomics, Princeton University, 132 Carl C. Icahn Laboratory, Princeton University, Washington Road, Princeton NJ 08540, USA.

Abstract

BACKGROUND:

Trimethoprim is a widely prescribed antibiotic for a variety of bacterial infections. It belongs to a class of anti-metabolites - antifolates - which includes drugs used against malarial parasites and in cancer therapy. However, spread of bacterial resistance to the drug has severely hampered its clinical use and has necessitated further investigations into its mechanism of action and treatment regimen. Trimethoprim selectively starves bacterial cells for tetrahydrofolate, a vital cofactor necessary for the synthesis of several metabolites. The outcome (bacteriostatic or bactericidal) of such starvation, however, depends on the availability of folate-dependent metabolites in the growth medium. To characterize this dependency, we investigated in detail the regulatory and structural components of Escherichia coli cellular response to trimethoprim in controlled growth and supplementation conditions.

RESULTS:

We surveyed transcriptional responses to trimethoprim treatment during bacteriostatic and bactericidal conditions and analyzed associated gene sets/pathways. Concurrent starvation of all folate dependent metabolites caused growth arrest, and this was accompanied by induction of general stress and stringent responses. Three gene sets were significantly associated with the bactericidal effect of TMP in different media including LB: genes of the SOS regulon, genes of the pyrimidine nucleotide biosynthetic pathway and members of the multiple antibiotic resistance (mar) regulon controlled by the MarR repressor. However, the SOS response was identified as the only universal transcriptional signature associated with the loss of viability by direct thymine starvation or by folate stress. We also used genome-wide gene knock-out screen to uncover means of sensitization of bacteria to the drug. We observed that among a number of candidate genes and pathways, the effect of knock-outs in the deoxyribose nucleotide salvage pathway, encoded by the deoCABD operon and under the control of the DeoR repressor, was most informative.

CONCLUSION:

Transcriptional induction of DNA damage response is an essential feature of the bactericidal effect of trimethoprim. Either the observation of the transcriptional response or DNA damage itself, or both, is made possible by thymine starvation when other folate-dependent metabolites are not limited. The effect of DNA damage by the drug takes place prior to its bactericidal effect, at the beginning of the lag stage of the treatment. Mutations in the deoxyribose nucleotide salvage pathway can affect duration of the lag as well as the rate of

killing. This information can be used to postulate certain mechanistic differences between direct thymine starvation in thymidylate synthase deficient mutants and thymine starvation by anti-folate inhibitors.

Supplemental Content

PLoS Pathog. 2012 Feb;8(2):e1002505. Epub 2012 Feb 2.

Genetic pathway in acquisition and loss of vancomycin resistance in a methicillin resistant Staphylococcus aureus (MRSA) strain of clonal type USA300.

Gardete S, Kim C, Hartmann BM, Mwangi M, Roux CM, Dunman PM, Chambers HF, Tomasz A.

Source

Laboratory of Microbiology, The Rockefeller University, New York, New York, USA.

Abstract

An isolate of the methicillin-resistant Staphylococcus aureus (MRSA) clone USA300 with reduced susceptibility to vancomycin (SG-R) (i.e, vancomycin-intermediate S. aureus, VISA) and its susceptible "parental" strain (SG-S) were recovered from a patient at the end and at the beginning of an unsuccessful vancomycin therapy. The VISA phenotype was unstable in vitro generating a susceptible revertant strain (SG-rev). The availability of these 3 isogenic strains allowed us to explore genetic correlates of antibiotic resistance as it emerged in vivo. Compared to the susceptible isolate, both the VISA and revertant strains carried the same point mutations in yycH, vraG, yvqF and lspA genes and a substantial deletion within an intergenic region. The revertant strain carried a single additional frameshift mutation in vraS which is part of two component regulatory system VraSR. VISA isolate SG-R showed complex alterations in phenotype: decreased susceptibility to other antibiotics, slow autolysis, abnormal cell division and increased thickness of cell wall. There was also altered expression of 239 genes including down-regulation of major virulence determinants. All phenotypic properties and gene expression profile returned to parental levels in the revertant strain. Introduction of wild type yvqF on a multicopy plasmid into the VISA strain caused loss of resistance along with loss of all the associated phenotypic changes. Introduction of the wild type vraSR into the revertant strain caused recovery of VISA type resistance. The yvqF/vraSR operon seems to function as an on/off switch: mutation in yvqF in strain SG-R turns on the vraSR system, which leads to increase in vancomycin resistance and down-regulation of virulence determinants. Mutation in vraS in the revertant strain turns off this regulatory

system accompanied by loss of resistance and normal expression of virulence genes. Down-regulation of virulence genes may provide VISA strains with a "stealth" strategy to evade detection by the host immune system.

Supplemental Content

Antimicrob Agents Chemother. 2012 Apr;56(4):1845-53. Epub 2012 Jan 30.

Deletion of TnAbaR23 results in both expected and unexpected antibiogram changes in a multidrug-resistant Acinetobacter baumannii strain.

Kochar M, Crosatti M, Harrison EM, Rieck B, Chan J, Constantinidou C, Pallen M, Ou HY, Rajakumar K.

Source

Department of Infection, Immunity and Inflammation, University of Leicester, Leicester, United Kingdom.

Abstract

Since the 2006 discovery of the Acinetobacter baumannii strain AYE AbaR1 resistance island, similar elements have been reported in numerous members of this species. As AbaR1 is distantly related to Tn7, we have renamed it TnAbaR1. TnAbaR transposons are known to carry multiple antibiotic resistance- and efflux-associated genes, although none have been experimentally studied en bloc. We deleted the TnAbaR transposon in A. baumannii A424, which we have designated TnAbaR23, and characterized independent deletion mutants DCO163 and DCO174. The NotI pulsed-field gel electrophoresis (PFGE) profile of strain DCO174 was consistent with targeted deletion of TnAbaR23 alone, but strain DCO163 apparently harbored a second large genomic deletion. Nevertheless, "subtractive amplification" targeting 52 TnAbaR and/or resistance-associated loci yielded identical results for both mutants and highlighted genes lost relative to strain A424. PCR mapping and genome sequencing revealed the entire 48.3-kb sequence of TnAbaR23. Consistent with TnAbaR23 carrying two copies of sul1, both mutants exhibited markedly increased susceptibility to sulfamethoxazole. In contrast, loss of tetAR(A) resulted in only a minor and variable increase in tetracycline susceptibility. Despite not exhibiting a growth handicap, strain DCO163 was more susceptible than strain DCO174 to 9 of 10 antibiotics associated with mutant-to-mutant variation in susceptibility, suggesting impairment of an undefined resistance-associated function. Remarkably, despite all three strains sharing identical gyrA and parC sequences, the ciprofloxacin MIC of DCO174 was >8-fold that of DCO163 and A424, suggesting a possible paradoxical role for TnAbaR23 in promoting sensitivity to ciprofloxacin. This study

highlights the importance of experimental scrutiny and challenges the assumption that resistance phenotypes can reliably be predicted from genotypes alone.

Supplemental Content

BMC Infect Dis. 2012 Jan 20;12:9.

Drug resistance mutations and heteroresistance detected using the GenoType MTBDRplus assay and their implication for treatment outcomes in patients from Mumbai, India.

Tolani MP, D'souza DT, Mistry NF.

Source

The Foundation for Medical Research, Worli, Mumbai, India.

Abstract

BACKGROUND:

Only 5% of the estimated global multidrug resistant TB (MDRTB) load is currently detected. Endemic Mumbai with increasing MDR would benefit from the introduction of molecular methods to detect resistance.

METHODS:

The GenoType MTBDRplus assay was used to determine mutations associated with isoniazid and rifampicin resistance and their correlation with treatment outcomes. It was performed on a convenience sample comprising 88 onset and 67 fifth month isolates for which phenotypic drug susceptibility testing (DST) was determined by the Buddemeyer technique for an earlier study. Simultaneous presence of wild type and mutant bands was referred to as "mixed patterns" (heteroresistance).

RESULTS:

Phenotypically 41 isolates were sensitive; 11 isoniazid, 2 rifampicin, 2 pyrazinamide and 5 ethambutol monoresistant; 16 polyresistant and 78 MDR. The agreement between both methods was excellent (kappa = 0.72-0.92). Of 22 rifampicin resistant onset isolates, the predominant rpoB mutations were the singular lack of WT8 (n = 8) and mixed D516V patterns (n = 9). Of the 64 rifampicin resistant fifth month isolates, the most frequent

mutations were in WT8 (n = 31) with a further 9 showing the S531L mutation. Mixed patterns were seen in 22 (34%) isolates, most frequently for the D516V mutation (n = 21). Of the 22 onset and 35 fifth month katG mutants, 13 and 12 respectively showed the S315T1 mutation with loss of the WT. Mixed patterns involving both S315T1 and S315T2 were seen in 9 and 23 isolates respectively. Seventeen of 23 and 23/35 inhA mutant onset and fifth month isolates showed mixed A16G profiles. Additionally, 10 fifth month isolates lacked WT2. Five onset and 6 fifth month isolates had both katG and inhA mutations. An association was noted between only katG but not only inhA resistance and poor outcome (p = 0.037); and additional resistance to ethambutol (p = 0.0033). More fifth month than onset isolates had mixed profiles for at least 1 gene (p = 0.000001).

CONCLUSIONS:

The use of the assay to rapidly diagnose MDR could guide simultaneous first- and second-line DST, and reduce the delay in administering appropriate regimens. Furthermore, detection of heteroresistance could prevent inaccurate "cured" treatment outcomes documented through smear microscopy and permit more sensitive detection of neonascent resistance.

Supplemental Content

Antimicrob Agents Chemother. 2012 Apr;56(4):2074-83. Epub 2012 Jan 9.

Functional and genetic characterization of the tap efflux pump in Mycobacterium bovis BCG.

Ramón-García S, Mick V, Dainese E, Martín C, Thompson CJ, De Rossi E, Manganelli R, Aínsa JA.

Source

Departamento de Microbiología, Medicina Preventiva y Salud Pública, Universidad de Zaragoza, Zaragoza, Spain. ramon@mail.ubc.ca

Abstract

Efflux pumps extrude a wide variety of chemically unrelated compounds conferring multidrug resistance and participating in numerous physiological processes. Mycobacterium tuberculosis possesses many efflux pumps, and their roles in drug resistance and physiology are actively investigated. In this work we found that tap mutant cells showed changes in morphology and a progressive loss of viability upon subcultivation in liquid medium. Transcriptome analysis in Mycobacterium bovis BCG revealed that disruption of the Rv1258c gene, encoding the Tap efflux pump, led to an extensive change in gene expression patterns during stationary phase, with no changes during exponential growth. In stationary phase, Tap inactivation triggered a general stress response and led to a general repression of genes involved in cell wall

biosynthesis, in particular the formation of the peptidoglycan; this suggested the accumulation of an unknown Tap substrate that reaches toxic concentrations during stationary phase. We also found that both disruption and overexpression of tap altered susceptibility to many clinically approved antibiotics in M. bovis BCG. Acriflavine and tetracycline accumulation assays and carbonyl cyanide m-chlorophenylhydrazone (CCCP) potentiation experiments demonstrated that this phenotype was due to an active efflux mechanism. These findings emphasize the important role of the Tap efflux pump in bacterial physiology and intrinsic drug resistance.

Supplemental Content

Appl Environ Microbiol. 2012 Feb;78(4):1004-14. Epub 2011 Dec 16.

Evolutionary silence of the acid chaperone protein HdeB in enterohemorrhagic Escherichia coli O157:H7.

Carter MQ, Louie JW, Fagerquist CK, Sultan O, Miller WG, Mandrell RE.

Source

Produce Safety and Microbiology Research Unit, Western Regional Research Center, Agricultural Research Service, US Department of Agriculture, Albany, California, USA. michelle.carter@ars.usda.gov

Abstract

The periplasmic chaperones HdeA and HdeB are known to be important for cell survival at low pH (pH < 3) in Escherichia coli and Shigella spp. Here we investigated the roles of HdeA and HdeB in the survival of various enterohemorrhagic E. coli (EHEC) following exposure to pH 2.0. Similar to K-12 strains, the acid protections conferred by HdeA and HdeB in EHEC O145 were significant: loss of HdeA and HdeB led to over 100- to 1,000-fold reductions in acid survival, depending on the growth condition of prechallenge cells. However, this protection was much less in E. coli O157:H7 strains. Deletion of hdeB did not affect the acid survival of cells, and deletion of hdeA led to less than a 5-fold decrease in survival. Sequence analysis of the hdeAB operon revealed a point mutation at the putative start codon of the hdeB gene in all 26 E. coli O157:H7 strains analyzed, which shifted the ATG start codon to ATA. This mutation correlated with the lack of HdeB in E. coli O157:H7; however, the plasmid-borne O157-hdeB was able to restore partially the acid resistance in an E. coli O145ΔhdeAB mutant, suggesting the potential function of O157-HdeB as an acid chaperone. We conclude that E. coli O157:H7 strains have evolved acid survival strategies independent of the HdeA/B chaperones and are more acid resistant than nonpathogenic K-12 for cells grown under nonfavorable culturing conditions such as in Luria-Bertani no-salt broth at 28°C. These results suggest a divergent evolution of acid resistance mechanisms within E. coli.

BMC Genomics. 2011 Nov 28;12:583.

The association of DNA damage response and nucleotide level modulation with the antibacterial mechanism of the anti-folate drug trimethoprim.

Sangurdekar DP, Zhang Z, Khodursky AB.

Source

Lewis-Sigler Institute for Integrative Genomics, Princeton University, 132 Carl C. Icahn Laboratory, Princeton University, Washington Road, Princeton NJ 08540, USA.

Abstract

BACKGROUND:

Trimethoprim is a widely prescribed antibiotic for a variety of bacterial infections. It belongs to a class of anti-metabolites - antifolates - which includes drugs used against malarial parasites and in cancer therapy. However, spread of bacterial resistance to the drug has severely hampered its clinical use and has necessitated further investigations into its mechanism of action and treatment regimen. Trimethoprim selectively starves bacterial cells for tetrahydrofolate, a vital cofactor necessary for the synthesis of several metabolites. The outcome (bacteriostatic or bactericidal) of such starvation, however, depends on the availability of folate-dependent metabolites in the growth medium. To characterize this dependency, we investigated in detail the regulatory and structural components of Escherichia coli cellular response to trimethoprim in controlled growth and supplementation conditions.

RESULTS:

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nucleotide salvage pathway, encoded by the deoCABD operon and under the control of the DeoR repressor, was most informative.

CONCLUSION:

Transcriptional induction of DNA damage response is an essential feature of the bactericidal effect of trimethoprim. Either the observation of the transcriptional response or DNA damage itself, or both, is made possible by thymine starvation when other folate-dependent metabolites are not limited. The effect of DNA damage by the drug takes place prior to its bactericidal effect, at the beginning of the lag stage of the treatment. Mutations in the deoxyribose nucleotide salvage pathway can affect duration of the lag as well as the rate of killing. This information can be used to postulate certain mechanistic differences between direct thymine starvation in thymidylate synthase deficient mutants and thymine starvation by anti-folate inhibitors.

Supplemental Content

Antimicrob Agents Chemother. 2012 Feb;56(2):1019-30. Epub 2011 Nov 21.

PmrB mutations promote polymyxin resistance of Pseudomonas aeruginosa isolated from colistin-treated cystic fibrosis patients.

Moskowitz SM, Brannon MK, Dasgupta N, Pier M, Sgambati N, Miller AK, Selgrade SE, Miller SI, Denton M, Conway SP, Johansen HK, Høiby N.

Source

Department of Pediatrics, Massachusetts General Hospital, Boston, Massachusetts, USA. smoskowitz@partners.org

Abstract

Pseudomonas aeruginosa can develop resistance to polymyxin and other cationic antimicrobial peptides. Previous work has shown that mutations in the PmrAB and PhoPQ regulatory systems can confer low to moderate levels of colistin (polymyxin E) resistance in laboratory strains and clinical isolates of this organism (MICs of 8 to 64 mg/liter). To explore the role of PmrAB in high-level clinical polymyxin resistance, P. aeruginosa isolates from chronically colistin-treated cystic fibrosis patients, most with colistin MICs of >512 mg/liter, were analyzed. These cystic fibrosis isolates contained probable gain-of-function pmrB alleles that conferred polymyxin resistance to strains with a wild-type or pmrAB deletion background. Double mutant pmrB alleles that contained mutations in both the periplasmic and dimerization-phosphotransferase domains markedly augmented polymyxin resistance.

Expression of mutant pmrB alleles induced transcription from the promoter of the arnB operon and stimulated addition of 4-amino-l-arabinose to lipid A, consistent with the known role of this lipid A modification in polymyxin resistance. For some highly polymyxin-resistant clinical isolates, repeated passage without antibiotic selection pressure resulted in loss of resistance, suggesting that secondary suppressors occur at a relatively high frequency and account for the instability of this phenotype. These results indicate that pmrB gain-of-function mutations can contribute to high-level polymyxin resistance in clinical strains of P. aeruginosa.

Supplemental Content

J Bacteriol. 2012 Jan;194(2):413-25. doi: 10.1128/JB.05864-11. Epub 2011 Nov 11.

Loss of elongation factor P disrupts bacterial outer membrane integrity.

Zou SB, Hersch SJ, Roy H, Wiggers JB, Leung AS, Buranyi S, Xie JL, Dare K, Ibba M, Navarre WW.

Source

Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Erratum in

- J Bacteriol. 2012 Aug;194(16):4484.

Abstract

Elongation factor P (EF-P) is posttranslationally modified at a conserved lysyl residue by the coordinated action of two enzymes, PoxA and YjeK. We have previously established the importance of this modification in Salmonella stress resistance. Here we report that, like poxA and yjeK mutants, Salmonella strains lacking EF-P display increased susceptibility to hypoosmotic conditions, antibiotics, and detergents and enhanced resistance to the compound S-nitrosoglutathione. The susceptibility phenotypes are largely explained by the enhanced membrane permeability of the efp mutant, which exhibits increased uptake of the hydrophobic dye 1-N-phenylnaphthylamine (NPN). Analysis of the membrane proteomes of wild-type and efp mutant Salmonella strains reveals few changes, including the prominent overexpression of a single porin, KdgM, in the efp mutant outer membrane. Removal of KdgM in the efp mutant background ameliorates the detergent, antibiotic, and osmosensitivity phenotypes and restores wild-type permeability to NPN. Our data support a role for EF-P in the translational regulation of a limited number of proteins that, when perturbed, renders the cell susceptible to stress by the adventitious overexpression of an outer membrane porin.

Antimicrob Agents Chemother. 2012 Jan;56(1):59-69. Epub 2011 Oct 24.

Colistin-resistant, lipopolysaccharide-deficient Acinetobacter baumannii responds to lipopolysaccharide loss through increased expression of genes involved in the synthesis and transport of lipoproteins, phospholipids, and poly-β-1,6-N-acetylglucosamine.

Henry R, Vithanage N, Harrison P, Seemann T, Coutts S, Moffatt JH, Nation RL, Li J, Harper M, Adler B, Boyce JD.

Source

Department of Microbiology, Monash University, Clayton, Australia.

Abstract

We recently demonstrated that colistin resistance in Acinetobacter baumannii can result from mutational inactivation of genes essential for lipid A biosynthesis (Moffatt JH, et al., Antimicrob. Agents Chemother. 54:4971-4977). Consequently, strains harboring these mutations are unable to produce the major Gram-negative bacterial surface component, lipopolysaccharide (LPS). To understand how A. baumannii compensates for the lack of LPS, we compared the transcriptional profile of the A. baumannii type strain ATCC 19606 to that of an isogenic, LPS-deficient, lpxA mutant strain. The analysis of the expression profiles indicated that the LPS-deficient strain showed increased expression of many genes involved in cell envelope and membrane biogenesis. In particular, upregulated genes included those involved in the Lol lipoprotein transport system and the Mla-retrograde phospholipid transport system. In addition, genes involved in the synthesis and transport of poly-β-1,6-N-acetylglucosamine (PNAG) also were upregulated, and a corresponding increase in PNAG production was observed. The LPS-deficient strain also exhibited the reduced expression of genes predicted to encode the fimbrial subunit FimA and a type VI secretion system (T6SS). The reduced expression of genes involved in T6SS correlated with the detection of the T6SS-effector protein AssC in culture supernatants of the A. baumannii wild-type strain but not in the LPS-deficient strain. Taken together, these data show that, in response to total LPS loss, A. baumannii alters the expression of critical transport and biosynthesis systems associated with modulating the composition and structure of the bacterial surface.

Supplemental Content

Antimicrob Agents Chemother. 2012 Jan;56(1):579-81. Epub 2011 Oct 10.

Loss of function of the gdpP protein leads to joint β-lactam/glycopeptide tolerance in Staphylococcus aureus.

Griffiths JM, O'Neill AJ.

Source

Antimicrobial Research Centre and Institute of Molecular and Cellular Biology, University of Leeds, Leeds, United Kingdom.

Abstract

The genetic basis of tolerance to inhibitors of peptidoglycan biosynthesis in Staphylococcus aureus was investigated by generating tolerant mutants in vitro and characterizing them by comparative genome sequencing. Two independently selected tolerant mutants harbored nonsynonymous mutations in gdpP, a gene encoding a putative membrane-located signaling protein. Insertional inactivation of gdpP also conferred tolerance. Our findings further implicate altered signal transduction as a route to antibiotic tolerance in S. aureus.

Supplemental Content

Proc Natl Acad Sci U S A. 2011 Oct 11;108(41):17165-70. Epub 2011 Oct 3.

Antimicrobial resistance to ceftazidime involving loss of penicillin-binding protein 3 in Burkholderia pseudomallei.

Chantratita N, Rholl DA, Sim B, Wuthiekanun V, Limmathurotsakul D, Amornchai P, Thanwisai A, Chua HH, Ooi WF, Holden MT, Day NP, Tan P, Schweizer HP, Peacock SJ.

Source

Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand.

Abstract

Known mechanisms of resistance to β-lactam antibiotics include β-lactamase expression, altered drug target, decreased bacterial permeability, and increased drug efflux. Here, we describe a unique mechanism of β-lactam resistance in the biothreat organism Burkholderia pseudomallei (the cause of melioidosis), associated with treatment failure during prolonged ceftazidime therapy of natural infection. Detailed comparisons of the initial ceftazidime-susceptible infecting isolate and subsequent ceftazidime-resistant variants from six patients led us to identify a common, large-scale genomic loss involving a minimum of 49 genes in all six resistant strains. Mutational analysis of wild-type B. pseudomallei demonstrated that ceftazidime resistance was due to deletion of a gene encoding a penicillin-binding protein 3 (BPSS1219) present within the region of genomic loss. The clinical ceftazidime-resistant variants failed to grow using commonly used laboratory culture media, including commercial blood cultures, rendering the variants almost undetectable in the diagnostic laboratory. Melioidosis is notoriously difficult to cure and clinical treatment failure is common in patients treated with ceftazidime, the drug of first choice across most of Southeast Asia where the majority of cases are reported. The mechanism described here represents an explanation for ceftazidime treatment failure, and may be a frequent but undetected resistance event.

Supplemental Content

J Bacteriol. 2011 Dec;193(23):6712-23. Epub 2011 Sep 30.

Hopanoid production is required for low-pH tolerance, antimicrobial resistance, and motility in Burkholderia cenocepacia.

Schmerk CL, Bernards MA, Valvano MA.

Source

Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, N6A 5C1, Canada.

Abstract

Hopanoids are pentacyclic triterpenoids that are thought to be bacterial surrogates for eukaryotic sterols, such as cholesterol, acting to stabilize membranes and to regulate their fluidity and permeability. To date, very few studies have evaluated the role of hopanoids in bacterial physiology. The synthesis of hopanoids depends on the enzyme squalene-hopene cyclase (Shc), which converts the linear squalene into the basic hopene structure. Deletion of the 2 genes encoding Shc enzymes in Burkholderia cenocepacia K56-2, BCAM2831 and BCAS0167, resulted in a strain that was unable to produce hopanoids, as demonstrated by gas chromatography and mass spectrometry. Complementation of the Δshc mutant with only BCAM2831 was sufficient to restore hopanoid production to wild-type levels, while

introducing a copy of BCAS0167 alone into the Δshc mutant produced only very small amounts of the hopanoid peak. The Δshc mutant grew as well as the wild type in medium buffered to pH 7 and demonstrated no defect in its ability to survive and replicate within macrophages, despite transmission electron microscopy (TEM) revealing defects in the organization of the cell envelope. The Δshc mutant displayed increased sensitivity to low pH, detergent, and various antibiotics, including polymyxin B and erythromycin. Loss of hopanoid production also resulted in severe defects in both swimming and swarming motility. This suggests that hopanoid production plays an important role in the physiology of B. cenocepacia.

Supplemental Content

Antimicrob Agents Chemother. 2011 Jun;55(6):2506-14. Epub 2011 Mar 21.

Loss of heterozygosity of FCY2 leading to the development of flucytosine resistance in Candida tropicalis.

Chen YN, Lo HJ, Wu CC, Ko HC, Chang TP, Yang YL.

Source

Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan, Republic of China.

Abstract

As fluconazole resistance becomes an emerging issue for treating infections caused by Candida tropicalis, searching for alternative becomes a prominent task. In the present study, 97 clinical isolates of C. tropicalis were tested for the susceptibilities to flucytosine (5FC) with the Etest method. Although only one isolate was resistant to 5FC, 30 susceptible isolates could produce resistant progeny after exposure to the drug. Interestingly, 22 of these 30 clinical isolates had a heterozygous G/T at the 145th position on FCY2, encoding purine-cytosine permease, whereas their progeny recovered from within the inhibitory ellipses had homozygous T/T, resulting in null alleles for both copies of the gene and produced only truncated proteins, effecting the 5FC resistance. Furthermore, we found that two major fluconazole-resistant clinical clones, diploid sequence type 98 (DST98) and DST140, had a homozygous G/G at the 145th position, and neither was able to produce 5FC-resistant progeny within the inhibitory ellipses. Hence, strains of C. tropicalis containing heterozygous alleles may develop 5FC resistance readily, whereas those with homozygous G/G wild-type alleles can be treated with 5FC. Subsequently, a combination of 5FC and another antifungal drug is applicable for treating infections of C. tropicalis.

Supplemental Content

Mol Microbiol. 2011 Jan;79(2):279-82. doi: 10.1111/j.1365-2958.2010.07459.x. Epub 2010 Nov 21.

The joys and terrors of fast adaptation: new findings elucidate antibiotic resistance and natural selection.

Roth JR.

Source

Department of Microbiology, University of California, Davis, One Shields Ave, Davis, CA 95616, USA. jrroth@ucdavis.edu

Abstract

Experiments of Pränting and Andersson demonstrate how bacteria adapt to the growth limitation caused by antibiotic resistance mutations. The process of adaptation relies on gene copy number changes that arise at high rates, including duplications (10(-4) per cell per generation), amplifications (10(-2) per cell per generation) and mutant copy loss (10(-2) per cell per division). Reversible increases in copy number improve growth by small steps and provide more targets for rare sequence alterations (10(-9) per cell per division) that can stably improve growth. After sequence alteration, selection favours loss of the still mutant gene copies that accelerated adaptation. The results strongly support the amplification-reversion model for fast adaptation and argue against the alternative idea of 'stress-induced mutagenesis'.

Supplemental Content

Antimicrob Agents Chemother. 2011 Mar;55(3):1211-21. Epub 2010 Dec 13.

A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and β-lactams in Pseudomonas aeruginosa.

Muller C, Plésiat P, Jeannot K.

Source

Laboratoire de Bactériologie, Faculté de Médecine, Université de Franche-Comté, 25030 Besançon, France.

Abstract

Constitutive overexpression of the active efflux system MexXY/OprM is a major cause of resistance to aminoglycosides, fluoroquinolones, and cefepime in clinical strains of Pseudomonas aeruginosa. Upregulation of this pump often results from mutations occurring in mexZ, the local repressor gene of the mexXY operon. In this study, analysis of MexXY-overproducing mutants selected in vitro from reference strain PAO1Bes on amikacin (at a concentration 1.5-fold higher than the MIC) led to identification of a new class of mutants harboring an intact mexZ gene and exhibiting increased resistance to colistin and imipenem in addition to aminoglycosides, fluoroquinolones, and cefepime. Reverse transcription-quantitative PCR (RT-qPCR) experiments on a selected clone named PAOW2 demonstrated that mexXY overexpression was independent of mexZ and the PA5471 gene, which is required for drug-dependent induction of mexXY. Furthermore, the transcript levels of the oprD gene, which encodes the carbapenem-selective porin OprD, were found to be reduced drastically in PAOW2. Whole-genome sequencing revealed a single mutation resulting in an M59I substitution in the ParR protein, the response regulator of the ParRS two-component regulatory system (with ParS being the sensor kinase), which is required for adaptive resistance of P. aeruginosa to polycationic peptides such as colistin. The multidrug resistance phenotype was suppressed in PAOW2 by deletion of the parS and parRS genes and conferred to PAO1Bes by chromosomal insertion of the mutated parRS locus from PAOW2. As shown by transcriptomic analysis, only a very small number of genes were expressed differentially between PAOW2 and PAO1Bes, including the lipopolysaccharide (LPS) modification operon arnBCADTEF-ugd, responsible for resistance to polycationic agents. Exposure of wild-type PAO1Bes to different polycationic peptides, including colistin, was shown to result in increased mexY and repressed oprD expression via ParRS, independent of PA5471. In agreement with these results, colistin antagonized activity of the MexXY/OprM substrates in PAO1Bes but not in a ΔparRS derivative. Finally, screening of clinical strains exhibiting the PAOW2 resistance phenotype allowed the identification of additional alterations in ParRS. Collectively, our data indicate that ParRS may promote either induced or constitutive multidrug resistance to four different classes of antibiotics through the activation of three distinct mechanisms (efflux, porin loss, and LPS modification).

Supplemental Content

Med Sci (Paris). 2010 Nov;26(11):960-8.

[Multi-drug resistant Pseudomonas aeruginosa: towards a therapeutic dead end?].

[Article in French]
Barbier F, Wolff M.

Source

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francois.barbier@bch.aphp.fr

Abstract

Pseudomonas aeruginosa is a major hospital-associated pathogen that can cause severe infections, most notably in patients with cystic fibrosis or those hospitalized in intensive care units. In this context, the current increase in incidence of multi-drug resistant (MDR) isolates of P. aeruginosa (MDRPA) raises serious concerns. MDR in P. aeruginosa is defined as the resistance to 3 or 4 of the following antibiotic classes: penicillins/cephalosporins/monobactams, carbapenems, aminoglycosides, and fluoroquinolones. These strains constantly cumulate several resistance mechanisms as a consequence of multiple genetic events, i.e., chromosomal mutations or horizontal transfers of resistance genes. Involved mechanisms may include active efflux, impermeability resulting from porins loss, plasmid-encoded b-lactamases/carbapenemases or aminoglycosides-modifying enzymes, and enzymatic or mutation-associated changes in antibiotics targets. Antibiotic selection pressure represents the leading risk factor for MDRPA acquisition. Colistin (polymyxin E) remains active on virtually all MDRPA isolates, and increasingly appears as the last available option to treat infections caused by these strains. However, the emergence of colistin resistance has been reported in P. aeruginosa, which may announce the spread of pan-resistant strains in a close future.

Supplemental Content

MBio. 2010 Oct 12;1(4). pii: e00227-10.

Multidrug-resistant enterococci lack CRISPR-cas.

Palmer KL, Gilmore MS.

Source

Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA.

Abstract

Clustered, regularly interspaced short palindromic repeats (CRISPR) provide bacteria and archaea with sequence-specific, acquired defense against plasmids and phage. Because mobile elements constitute up to 25% of the genome of multidrug-resistant (MDR) enterococci, it

was of interest to examine the codistribution of CRISPR and acquired antibiotic resistance in enterococcal lineages. A database was built from 16 Enterococcus faecalis draft genome sequences to identify commonalities and polymorphisms in the location and content of CRISPR loci. With this data set, we were able to detect identities between CRISPR spacers and sequences from mobile elements, including pheromone-responsive plasmids and phage, suggesting that CRISPR regulates the flux of these elements through the E. faecalis species. Based on conserved locations of CRISPR and CRISPR-cas loci and the discovery of a new CRISPR locus with associated functional genes, CRISPR3-cas, we screened additional E. faecalis strains for CRISPR content, including isolates predating the use of antibiotics. We found a highly significant inverse correlation between the presence of a CRISPR-cas locus and acquired antibiotic resistance in E. faecalis, and examination of an additional eight E. faecium genomes yielded similar results for that species. A mechanism for CRISPR-cas loss in E. faecalis was identified. The inverse relationship between CRISPR-cas and antibiotic resistance suggests that antibiotic use inadvertently selects for enterococcal strains with compromised genome defense.

Supplemental Content

J Med Microbiol. 2011 Feb;60(Pt 2):147-56. Epub 2010 Oct 21.

Co-regulation of {beta}-lactam resistance, alginate production and quorum sensing in Pseudomonas aeruginosa.

Balasubramanian D, Kong KF, Jayawardena SR, Leal SM, Sautter RT, Mathee K.

Source

Department of Biological Sciences, College of Arts and Science, Florida International University, Miami, FL 33199, USA.

Erratum in

- J Med Microbiol. 2011 May;60(Pt 5):696-7.

Abstract

Development of β-lactam resistance, production of alginate and modulation of virulence factor expression that alters host immune responses are the hallmarks of chronic Pseudomonas aeruginosa infection in cystic fibrosis patients. In this study, we propose that a co-regulatory network exists between these mechanisms. We compared the promoter activities of ampR, algT/U, lasR, lasI, rhlR, rhlI and lasA genes, representing the β-lactam antibiotic resistance master regulatory gene, the alginate switch operon, the las and rhl quorum-sensing (QS) genes, and the LasA staphylolytic protease, respectively. Four isogenic P. aeruginosa strains, the prototypic Alg(-) PAO1, Alg(-) PAOampR, the mucoid Alg(+)

PAOmucA22 (Alg(+) PDO300) and Alg(+) PAOmucA22ampR (Alg(+) PDOampR) were used. We found that in the presence of AmpR regulator and β-lactam antibiotic, the extracytoplasmic function sigma factor AlgT/U positively regulated P(ampR), whereas AmpR negatively regulated P(algT/U). On the basis of this finding we suggest the presence of a negative feedback loop to limit algT/U expression. In addition, the functional AlgT/U caused a significant decrease in the expression of QS genes, whereas loss of ampR only resulted in increased P(lasI) and P(lasR) transcription. The upregulation of the las QS system is likely to be responsible for the increased lasA promoter and the LasA protease activities in Alg(-) PAOampR and Alg(+) PDOampR. The enhanced expression of virulence factors in the ampR strains correlated with a higher rate of Caenorhabditis elegans paralysis. Hence, this study shows that the loss of ampR results in increased virulence, and is indicative of the existence of a co-regulatory network between β-lactam resistance, alginate production, QS and virulence factor production, with AmpR playing a central role.

Supplemental Content

Antimicrob Agents Chemother. 2010 Dec;54(12):4971-7. Epub 2010 Sep 20.

Colistin resistance in Acinetobacter baumannii is mediated by complete loss of lipopolysaccharide production.

Moffatt JH, Harper M, Harrison P, Hale JD, Vinogradov E, Seemann T, Henry R, Crane B, St Michael F, Cox AD, Adler B, Nation RL, Li J, Boyce JD.

Source

Department of Microbiology, Building 76, Monash University, Clayton, Victoria 3800, Australia.

Abstract

Infections caused by multidrug-resistant (MDR) Gram-negative bacteria represent a major global health problem. Polymyxin antibiotics such as colistin have resurfaced as effective last-resort antimicrobials for use against MDR Gram-negative pathogens, including Acinetobacter baumannii. Here we show that A. baumannii can rapidly develop resistance to polymyxin antibiotics by complete loss of the initial binding target, the lipid A component of lipopolysaccharide (LPS), which has long been considered to be essential for the viability of Gram-negative bacteria. We characterized 13 independent colistin-resistant derivatives of A. baumannii type strain ATCC 19606 and showed that all contained mutations within one of the first three genes of the lipid A biosynthesis pathway: lpxA, lpxC, and lpxD. All of these mutations resulted in the complete loss of LPS production. Furthermore, we showed that loss of LPS occurs in a colistin-resistant clinical isolate of A. baumannii. This is the first report of a spontaneously occurring, lipopolysaccharide-deficient, Gram-negative bacterium.

Clin Exp Immunol. 2008 Aug;153(2):214-20. Epub 2008 May 26.

Immunodeficiency in ataxia telangiectasia is correlated strongly with the presence of two null mutations in the ataxia telangiectasia mutated gene.

Staples ER, McDermott EM, Reiman A, Byrd PJ, Ritchie S, Taylor AM, Davies EG.

Source

Department of Immunology, Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham, UK.

Abstract

Immunodeficiency affects over half of all patients with ataxia telangiectasia (A-T) and when present can contribute significantly to morbidity and mortality. A retrospective review of clinical history, immunological findings, ataxia telangiectasia mutated (ATM) enzyme activity and ATM mutation type was conducted on 80 consecutive patients attending the National Clinic for Ataxia Telangiectasia, Nottingham, UK between 1994 and 2006. The aim was to characterize the immunodeficiency in A-T and determine its relationship to the ATM mutations present. Sixty-one patients had mutations resulting in complete loss of ATM kinase activity (group A) and 19 patients had leaky splice or missense mutations resulting in residual kinase activity (group B). There was a significantly higher proportion of patients with recurrent sinopulmonary infections in group A compared with group B (31 of 61 versus four of 19 P = 0.03) and a greater need for prophylactic antibiotics (30 of 61 versus one of 19 P = 0.001). Comparing group A with group B patients, 25 of 46 had undetectable/low immunoglobulin A (IgA) levels compared with none of 19; T cell lymphopenia was found in 28 of 56 compared with one of 18 and B cell lymphopenia in 35 of 55 compared with four of 18 patients (P = 0.00004, 0.001 and 0.003 respectively). Low IgG2 subclass levels and low levels of antibodies to pneumococcal polysaccharide were more common in group A than group B (16 of 27 versus one of 11 P = 0.01; 34/43 versus six of 17 P = 0.002) patients. Ig replacement therapy was required in 10 (12.5%) of the whole cohort, all in group A. In conclusion, A-T patients with no ATM kinase activity had a markedly more severe immunological phenotype than those expressing low levels of ATM activity.

Infect Immun. 1997 Apr;65(4):1395-401.

Effects of overexpression of the alkyl hydroperoxide reductase AhpC on the virulence and isoniazid resistance of Mycobacterium tuberculosis.

Heym B, Stavropoulos E, Honoré N, Domenech P, Saint-Joanis B, Wilson TM, Collins DM, Colston MJ, Cole ST.

Source

Unité de Génétique Moléculaire Bactérienne, Institut Pasteur, Paris, France.

Abstract

Mutations to the regulatory region of the ahpC gene, resulting in overproduction of alkyl hydroperoxide reductase, were encountered frequently in a large collection of isoniazid (INH)-resistant clinical isolates of Mycobacterium tuberculosis but not in INH-susceptible strains. Overexpression of ahpC did not seem to be important for INH resistance, however, as most of these strains were already defective for catalase-peroxidase, KatG, the enzyme required for activation of INH. Transformation of the INH-susceptible reference strain, M. tuberculosis H37Rv, with plasmids bearing the ahpC genes of M. tuberculosis or M. leprae did not result in a significant increase in the MIC. Two highly INH-resistant mutants of H37Rv, BH3 and BH8, were isolated in vitro and shown to produce no or little KatG activity and, in the case of BH3, to overproduce alkyl hydroperoxide reductase as the result of an ahpC regulatory mutation that was also found in some clinical isolates. The virulence of H37Rv, BH3, and BH8 was studied intensively in three mouse models: fully immunocompetent BALB/c and Black 6 mice, BALB/c major histocompatibility complex class II-knockout mice with abnormally low levels of CD4 T cells and athymic mice producing no cellular immune response. The results indicated that M. tuberculosis strains producing catalase-peroxidase were considerably more virulent in immunocompetent mice than the isogenic KatG-deficient mutants but that loss of catalase-peroxidase was less important when immunodeficient mice, unable to produce activated macrophages, were infected. Restoration of virulence was not seen in an INH-resistant M. tuberculosis strain that overexpressed ahpC, and this finding was confirmed by experiments performed with appropriate M. bovis strains in guinea pigs. Thus, in contrast to catalase-peroxidase, alkyl hydroperoxide reductase does not appear to act as a virulence factor in rodent infections or to play a direct role in INH resistance, although it may be important in maintaining peroxide homeostasis of the organism when KatG activity is low or absent.

Supplemental Content

Bacterial gene loss as a mechanism for gain of antimicrobial resistance.

Török M, Chantratita N, Peacock S.

Source

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Abstract

Acquisition of exogenous DNA by pathogenic bacteria represents the basis for much of the acquired antimicrobial resistance in pathogenic bacteria. A more extreme mechanism to avoid the effect of an antibiotic is to delete the drug target, although this would be predicted to be rare since drug targets are often essential genes. Here, we review and discuss the description of a novel mechanism of resistance to the cephalosporin drug ceftazidime caused by loss of a penicillin-binding protein (PBP) in a Gram-negative bacillus (Burkholderia pseudomallei). This organism causes melioidosis across south-east Asia and northern Australia, and is usually treated with two or more weeks of ceftazidime followed by oral antibiotics for three to six months. Comparison of clinical isolates from six patients with melioidosis found initial ceftazidime-susceptible isolates and subsequent ceftazidime-resistant variants. The latter failed to grow on commonly used culture media, rendering these isolates difficult to detect in the diagnostic laboratory. Genomic analysis using pulsed-field gel electrophoresis and array based genomic hybridisation revealed a large-scale genomic deletion comprising 49 genes in the ceftazidime-resistant strains. Mutational analysis of wild-type B. pseudomallei demonstrated that ceftazidime resistance was due to deletion of a gene encoding a PBP 3 present within the region of genomic loss. This provides one explanation for ceftazidime treatment failure, and may be a frequent but undetected event in patients with melioidosis.

Supplemental Content

Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species.

Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM.

Source

Centre of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Abstract

We have investigated to what extent natural transformation acting on free DNA substrates can facilitate transfer of mobile elements including transposons, integrons and/or gene cassettes between bacterial species. Naturally transformable cells of Acinetobacter baylyi were exposed to DNA from integron-carrying strains of the genera Acinetobacter, Citrobacter, Enterobacter, Escherichia, Pseudomonas, and Salmonella to determine the nature and frequency of transfer. Exposure to the various DNA sources resulted in acquisition of antibiotic resistance traits as well as entire integrons and transposons, over a 24 h exposure period. DNA incorporation was not solely dependent on integrase functions or the genetic relatedness between species. DNA sequence analyses revealed that several mechanisms facilitated stable integration in the recipient genome depending on the nature of the donor DNA; homologous or heterologous recombination and various types of transposition (Tn21-like and IS26-like). Both donor strains and transformed isolates were extensively characterized by antimicrobial susceptibility testing, integron- and cassette-specific PCRs, DNA sequencing, pulsed field gel electrophoreses (PFGE), Southern blot hybridizations, and by re-transformation assays. Two transformant strains were also genome-sequenced. Our data demonstrate that natural transformation facilitates interspecies transfer of genetic elements, suggesting that the transient presence of DNA in the cytoplasm may be sufficient for genomic integration to occur. Our study provides a plausible explanation for why sequence-conserved transposons, IS elements and integrons can be found disseminated among bacterial species. Moreover, natural transformation of integron harboring populations of competent bacteria revealed that interspecies exchange of gene cassettes can be highly efficient, and independent on genetic relatedness between donor and recipient. In conclusion, natural transformation provides a much broader capacity for horizontal acquisitions of genetic elements and hence, resistance traits from divergent species than previously assumed.

Supplemental Content

PLoS One. 2012;7(7):e42280. Epub 2012 Jul 31.

Analysis of drug resistance determinants in Klebsiella pneumoniae isolates from a tertiary-care hospital in Beijing, China.

Li B, Yi Y, Wang Q, Woo PC, Tan L, Jing H, Gao GF, Liu CH.

Source

Chinese Academy of Sciences Key Laboratory of Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Abstract

BACKGROUND:

The rates of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) isolates among Enterobacteriaceae isolates, particularly Klebsiella pneumoniae, have risen substantially worldwide.

METHODOLOGY/PRINCIPAL FINDINGS:

To better understand the molecular mechanisms of drug resistance in K. pneumoniae, we analyzed the drug resistance determinants for K. pneumoniae isolates collected from the 306 Hospital, a tertiary-care hospital in Beijing, China, for the period of September 1, 2010-October 31, 2011. Drug susceptibility testing, PCR amplification and sequencing of the drug resistance determinants were performed. Conjugation experiments were conducted to examine the natural ability of drug resistance to disseminate among Enterobacteriaceae strains using a sodium azide-resistant Escherichia coli J53 strain as a recipient. Among the 223 consecutive non-repetitive K. pneumoniae isolates included in this study, 101 (45.3%) were extended-spectrum beta-lactamases (ESBLs) positive. The rates of MDR, XDR, and PDR isolates were 61.4% (n = 137), 22.0% (n = 49), and 1.8% (n = 4), respectively. Among the tested drug resistance-associated genes, the following ones were detected at relatively high rates bla(CTX-M-10) (80, 35.9%), aacC2 (73, 32.7%), dhfr (62, 27.8%), qnrS (58, 26.0%), aacA4 (57, 25.6%), aadA1 (56, 25.1%). Results from conjugation experiments indicate that many of the drug resistance genes were transmissible.

CONCLUSIONS/SIGNIFICANCE:

Our data give a "snapshot" of the complex genetic background responsible for drug resistance in K. pneumoniae in China and demonstrate that a high degree of awareness and monitoring of those drug resistance determinants are urgently needed in order to better control the emergence and transmission of drug-resistant K. pneumoniae isolates in hospital settings.

Supplemental Content

MBio. 2012 Feb 21;3(1). pii: e00305-11. doi: 10.1128/mBio.00305-11. Print 2012.

Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock.

Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, Pearson T, Waters AE, Foster JT, Schupp J, Gillece J, Driebe E, Liu CM, Springer B, Zdovc I, Battisti A, Franco A, Zmudzki J, Schwarz S, Butaye P, Jouy E, Pomba C, Porrero MC, Ruimy R, Smith TC, Robinson DA, Weese JS, Arriola CS, Yu F, Laurent F, Keim P, Skov R, Aarestrup FM.

Source

Translational Genomics Research Institute (TGen), Pathogen Genomics Division, Flagstaff, Arizona, USA. lprice@tgen.org

Abstract

Since its discovery in the early 2000s, methicillin-resistant Staphylococcus aureus (MRSA) clonal complex 398 (CC398) has become a rapidly emerging cause of human infections, most often associated with livestock exposure. We applied whole-genome sequence typing to characterize a diverse collection of CC398 isolates (n = 89), including MRSA and methicillin-susceptible S. aureus (MSSA) from animals and humans spanning 19 countries and four continents. We identified 4,238 single nucleotide polymorphisms (SNPs) among the 89 core genomes. Minimal homoplasy (consistency index = 0.9591) was detected among parsimony-informative SNPs, allowing for the generation of a highly accurate phylogenetic reconstruction of the CC398 clonal lineage. Phylogenetic analyses revealed that MSSA from humans formed the most ancestral clades. The most derived lineages were composed predominantly of livestock-associated MRSA possessing three different staphylococcal cassette chromosome mec element (SCCmec) types (IV, V, and VII-like) including nine subtypes. The human-associated isolates from the basal clades carried phages encoding human innate immune modulators that were largely missing among the livestock-associated isolates. Our results strongly suggest that livestock-associated MRSA CC398 originated in humans as MSSA. The lineage appears to have undergone a rapid radiation in conjunction with the jump from humans to livestock, where it subsequently acquired tetracycline and methicillin resistance. Further analyses are required to estimate the number of independent genetic events leading to the methicillin-resistant sublineages, but the diversity of SCCmec subtypes is suggestive of strong and diverse antimicrobial selection associated with food animal production. IMPORTANCE: Modern food animal production is characterized by densely concentrated animals and routine antibiotic use, which may facilitate the emergence of novel antibiotic-resistant zoonotic pathogens. Our findings strongly support the idea that livestock-associated MRSA CC398 originated as MSSA in humans. The jump of CC398 from humans to livestock was accompanied by the loss of phage-carried human virulence genes, which likely attenuated its zoonotic potential, but it was also accompanied by the acquisition of tetracycline and methicillin resistance. Our findings exemplify a bidirectional zoonotic exchange and underscore the potential public health risks of widespread antibiotic use in food animal production.

Int Microbiol. 1998 Dec;1(4):265-70.

Bacterial evolution and the cost of antibiotic resistance.

Lenski RE.

Source

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Abstract

Bacteria clearly benefit from the possession of an antibiotic resistance gene when the corresponding antibiotic is present. But do resistant bacteria suffer a cost of resistance (i.e., a reduction in fitness) when the antibiotic is absent? If so, then one strategy to control the spread of resistance would be to suspend the use of a particular antibiotic until resistant genotypes declined to low frequency. Numerous studies have indeed shown that resistant genotypes are less fit than their sensitive counterparts in the absence of antibiotic, indicating a cost of resistance. But there is an important caveat: these studies have put resistance genes into naive bacteria, which have no evolutionary history of association with the resistance genes. An important question, therefore, is whether bacteria can overcome the cost of resistance by evolving adaptations that counteract the harmful side-effects of resistance genes. In fact, several experiments (in vitro and in vivo) show that the cost of antibiotic resistance can be substantially diminished, even eliminated, by evolutionary changes in bacteria over rather short periods of time. As a consequence, it becomes increasingly difficult to eliminate resistant genotypes simply by suspending the use of antibiotics.

Role of Reactive Oxygen Species in Antibiotic Action and Resistance

Daniel J Dwyer,[1] Michael A Kohanski,[1,2] and James J Collins[1,2,*]
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Abstract

The alarming spread of bacterial strains exhibiting resistance to current antibiotic therapies necessitates that we elucidate the specific genetic and biochemical responses underlying drug-mediated cell killing, so as to increase the efficacy of available treatments and develop new antibacterials. Recent research aimed at identifying such cellular contributions has revealed that antibiotics induce changes in metabolism that promote the formation of reactive oxygen species, which play a role in cell death. Here we discuss the relationship between drug-induced oxidative stress, the SOS response and their potential combined contribution to resistance development. Additionally, we describe ways in which these responses are being taken advantage of to combat bacterial infections and arrest the rise of

PLoS Pathog. 2012 Aug;8(8):e1002837. Epub 2012 Aug 2.

Natural transformation facilitates transfer of transposons, integrons and gene cassettes between bacterial species.

Domingues S, Harms K, Fricke WF, Johnsen PJ, da Silva GJ, Nielsen KM.

Source

Centre of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Abstract

We have investigated to what extent natural transformation acting on free DNA substrates can facilitate transfer of mobile elements including transposons, integrons and/or gene cassettes between bacterial species. Naturally transformable cells of Acinetobacter baylyi were exposed to DNA from integron-carrying strains of the genera Acinetobacter, Citrobacter, Enterobacter, Escherichia, Pseudomonas, and Salmonella to determine the nature and frequency of transfer. Exposure to the various DNA sources resulted in acquisition of antibiotic resistance traits as well as entire integrons and transposons, over a 24 h exposure period. DNA incorporation was not solely dependent on integrase functions or the genetic relatedness between species. DNA sequence analyses revealed that several mechanisms facilitated stable integration in the recipient genome depending on the nature of the donor DNA; homologous or heterologous recombination and various types of transposition (Tn21-like and IS26-like). Both donor strains and transformed isolates were extensively characterized by antimicrobial susceptibility testing, integron- and cassette-specific PCRs, DNA sequencing, pulsed field gel electrophoreses (PFGE), Southern blot hybridizations, and by re-transformation assays. Two

transformant strains were also genome-sequenced. Our data demonstrate that natural transformation facilitates interspecies transfer of genetic elements, suggesting that the transient presence of DNA in the cytoplasm may be sufficient for genomic integration to occur. Our study provides a plausible explanation for why sequence-conserved transposons, IS elements and integrons can be found disseminated among bacterial species. Moreover, natural transformation of integron harboring populations of competent bacteria revealed that interspecies exchange of gene cassettes can be highly efficient, and independent on genetic relatedness between donor and recipient. In conclusion, natural transformation provides a much broader capacity for horizontal acquisitions of genetic elements and hence, resistance traits from divergent species than previously assumed.

Supplemental Content

J Clin Microbiol. 2010 Jun;48(6):2016-21. Epub 2010 Mar 29.

High prevalence of dihydropteroate synthase mutations in Pneumocystis jirovecii isolated from patients with Pneumocystis pneumonia in South Africa.

Dini L, du Plessis M, Frean J, Fernandez V.

Source

Department of Parasitology, Mycology, and Environmental Microbiology, Swedish Institute for Infectious Disease Control, Solna, Sweden. leigh.dini@smi.se

Abstract

Pneumocystis jirovecii pneumonia (PCP) is an important cause of morbidity and mortality in immunocompromised patients. Sulfa-containing drugs are used for the treatment and prophylaxis of PCP. Mutations in the P. jirovecii fas gene, which encodes dihydropteroate synthase (DHPS), are associated with prior exposure to sulfa drugs, and their appearance suggests the emergence of variants with reduced sulfa susceptibility. The present study examined the prevalence of DHPS mutations in P. jirovecii strains isolated from South African patients with PCP. P. jirovecii infection was investigated by immunofluorescence microscopy and quantitative real-time PCR with respiratory specimens from 712 patients (93% of whom were >15 years of age) with suspected PCP consecutively received for the detection of P. jirovecii over 1 year. PCR amplification and sequencing of the DHPS fas gene was attempted with DNA from the P. jirovecii-positive samples. P. jirovecii infection was confirmed by immunofluorescence microscopy in 168/712 (24%) of the patients. Carriage of the fungus was revealed by real-time PCR in 17% of the patients with negative microscopy results. The P. jirovecii fas gene was successfully amplified from specimens from 151 patients and sequenced. Mutations resulting in the Thr55Ala and/or Pro57Ser amino acid substitution were detected in P. jirovecii strains from 85/151 (56%) patients. The high frequency of PCP

episodes with P. jirovecii harboring DHPS mutations in South Africa indicates that populations of this fungus are evolving under the considerable selective pressure exerted by sulfa-containing antibiotics. These results, similar to previous observations of sulfa drug resistance in bacterial populations, underscore the importance of the rational use of sulfa medications either prophylactically against PCP or for the treatment of other infections.

Supplemental Content

Med Sci Monit. 2011 Feb 25;17(3):CR154-8.

Confirmation of HIV-like sequences in respiratory tract bacteria of Cambodian and Kenyan HIV-positive pediatric patients.

Zajac V, Matelova L, Liskova A, Mego M, Holec V, Adamcikova Z, Stevurkova V, Shahum A, Krcmery V.

Source

Department of Cancer Genetics, Cancer Research Institute, Bratislava, Slovakia.

Abstract

BACKGROUND:

Bacteria and yeasts isolated from respiratory tracts of 39 Cambodian and 28 Kenyan HIV-positive children were tested for the presence of HIV-1 sequences.

MATERIAL/METHODS:

Bacteria and yeasts from the respiratory tract (nose, pharyngeal swabs) were isolated from 39 Cambodian and 28 Kenyan HIV-positive children. Bacterial chromosomal DNA was prepared by standard protocol and by Qiagen kit. The PCR specific for HIV sequences was carried out using HIV-1-specific primers.The analysis was performed by colony and dot-blot hybridization using HIV-1-specific primers which represent gag, pol and env genes of the virus. The sequencing of some PCR products was performed on the ABI 373 DNA Sequencer.

RESULTS:

The majority of microbes were characterized as Staphylococcus aureus, Klebsiella pneumoniae, and resp. Candida albicans. In some cases E. coli, Streptococcus pyogenes, Proteus mirabilis and Candida tropicalis were identified. Bacteria of 16 Cambodian (41%) and

8 Kenyan (31%) children were found to be positive in colony and dot-blot DNA hybridization. By the sequencing of PCR products synthesized on the template of patients' bacterial DNA using primers 68;69 for env HIV-1 gene, homology of greater than 90% with HIV-1 isolate HXB2 (HIVHXB2CG) was revealed.

CONCLUSIONS:

Bacteria and yeasts from the respiratory tract of 41% of Cambodian and 31% of Kenyan HIV-positive children bear HIV-like sequences. The role of bacteria in the HIV disease process is discussed.

Supplemental Content

The Role of Bacteria and Yeasts in AIDS

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http://www.intechopen.com/books/microbes-viruses-and-parasites-in-aids-process/the-role-of-bacteria-and-yeasts-in-aids