†1110 THE POTENTIAL FOR ORAL ANTIBIOTIC THERAPY FOR PSEUDOMONAS INFECTIONS IN CHILDREN. Melvin I. Marks, Stephen A. Chartrand, and Ronald Scribner, Univ. of Oklahoma Hlth. Sci. Ctr. and Univ. of South Alabama, Depts. of Pediatrics, Oklahoma City and Mobile.

New, orally absorbed quinoline derivatives are highly active against a broad spectrum of bacteria, including Pseudomonas (Antimicrob Ag Chemother 23:658, 1983). Pseudomonas (n=145) were tested in vitro against enoxacin, norfloxacin, ciprofloxacin, and ofloxacin. All were active against P. aeruginosa (MIC_{90} ≤4 µg/ml), including aminoglycoside-resistant isolates. Enoxacin and ciprofloxacin were most active against other Pseudomonas species, including many strains of P. cepacia and P. maltophilia. The pharmacokinetics of these drugs were determined in mice and rats and therapeutic studies performed in two models: (1) Graded inocula of Pseudomonas species were injected IP into mice, followed by single dose therapy ½ hour later. Oral enoxacin was more active than norfloxacin or tobramycin in reducing bacteremia and mortality due to P. aeruginosa and P. cepacia (e.g. LD_{50} 10^{2} for saline v. $10^{6.1}$ for enoxacin for P. aeruginosa); (2) Oral enoxacin was begun 3 days or 3 weeks after persistent Pseudomonas pulmonary infection in the agar-bead rat model. Bacterial concentrations in lung were reduced by 66% and 76%, respectively.

These in vitro and in vivo data support the continued development of quinolines as oral antibiotics for Pseudomonas infections in children.

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WITHDRAWN

1112 ANIMAL MODEL FOR TOXIC SHOCK SYNDROME (TSS). Marian E. Melish, Kanitha S. Frogner, Shirley S. Hirata, and Mieko S. Murata, University of Hawaii School of Medicine, Department of Pediatrics, Honolulu, Hawaii.

TSS Antigen (TSS Ag), the protein marker identifying TSS associated staphylococci, can be detected in blood, urine and tissues of patients with TSS but has not been proven to be the toxin responsible for shock and multisystem dysfunction. To assess pathogenicity we developed an animal model. Rabbits given I.V. bolus of 60 µg/kg of purified TSS Ag were unaffected but 100 µg/kg bolus dose → rapid ↑ in BUN, creatinine and SGOT, lymphopenia, profound hypotension by 12 hours and death by 24 hours. Baboons given 100 µg/kg by I.V. bolus showed no hypotension or chemical change but doses of 150-400 µg/kg given by infusion over 1-16 hours → azotemia, lymphopenia, moderate to profound hypotension in all and spontaneous mortality in 2/3. The profile of TSS Ag in serum and urine was followed by radioimmunoassay. In baboons TSS Ag was detected in serum immediately after 100 µg/kg bolus injection (ineffective dose) with peak concentration of >1300 ng/ml but was cleared rapidly. Infusion of 400 µg/kg over 4 hours → shock and death with peak concentration 110 ng/ml and persistence for 32 hours. Plasma levels of TSS Ag in rabbits and baboons with hypotension, azotemia, liver damage and lymphopenia are similar to those in experimental infection with TSS staphylococci (> 80 ng/ml sustained over several hours.) Autopsy resembled fatal human TSS. This animal model reproduces the major pathological features of TSS, suggesting that TSS Ag is the mediator of renal and hepatic dysfunction, shock and death either by direct action or through endogenous host response.

†1113 MOBILIZATION OF TYPE b CAPSULE GENE(S) IN HAEMOPHILUS INFLUENZAE b (Hib) BY CELL TO CELL CONTACT. P.M.Mendelman, V.Ph.Syriopoulou, S.L.Gandy, C.A. Doroshow, J. I.Ward, A.L.Smith. Univ. Wash., Child. Hosp., Dept. Peds.,Seattle.

Little is known about the genetics of the type b capsule (cap), the major virulence factor of Hib. By contrast, transfer of antibiotic resistance is well known to be mediated primarily by R-plasmids, though chromosomal gene transfer also occurs under conditions facilitating conjugation. During experiments to characterize plasmid-mediated Amp^R we mated 29 Amp^R Sm^S Alaskan Hib with an unencapsulated Amp^S Sm^R recipient. We noted large smooth cfus among the Amp^R transconjugants (TCs) in 25 of 29 matings. Testing these smooth strains for b cap. (antiserum agar and agglutination with anti b antiserum) revealed that all were type b. The background mutation freq. of the 25 donor strains to Sm^R was $\leq 10^{-9}$ and the mutation freq. of the unencapsulated recipient to produce type b cap. was undetectable ($<10^{-9}$). These data suggest that cap. expression was the result of gene transfer to the recipient and not a mutational event. To determine if this phenomenon occurs in other R-plasmid bearing HI, we mated an Amp, Cm and Te resistant Hib isolate with 2 different unencapsulated recipients. The type b cap. transferred with the antibiotic resistance determinants in a subset of TCs. Conjugal cap. transfer from this strain was not detected in the presence of DNAase, suggesting gene transfer occurred by transformation. We conclude that the type b cap. genes of these strains transfer concurrently with the plasmid as a common event in vitro. Whether the cap. b gene can be carried on an R plasmid or whether it is transferred by plasmid-facilitated chromosomal exchange remains to be determined.

1114 PENICILLIN BINDING PROTEINS (PBPs) IN HAEMOPHILUS INFLUENZAE (HI). P.M. Mendelman, D.O. Chaffin, V.Ph. Syriopoulou, J.Philpott-Howard, J.D. Williams, A.L. Smith. Univ. of Wash., Children's Orthopedic Hosp. and Med. Ctr., Dept. of Peds., Seattle and London Hosp. Med. Coll., England.

We previously described different PBP patterns of 3 non-typeable ampicillin-resistant (Amp^R)-β-lactamase-neg. HI. The resistance determinant could be mobilized by transformation permitting assessment and comparison of the PBPs in the Amp^R transformants (TFs) and the isogenic Amp^S recipient. PBPs were detected by incubation of crude membrane preparations with radiolabelled penicillin (PCN) followed by selective solubilization with sarkosyl, electrophoresis in SDS-PAGE and fluorography. Ten major PBPs ranging in mol. wt. from 88 to 27 Kd. were defined in the Amp^S recipient. Two TF strains showed apparent decreases in PBP 4 and 9. To measure the affinity of the PBPs for Amp, we assessed ^{3}H-PCN binding of the Amp^S recipient and 1 of these Amp^R TFs in the presence of Amp at serial ten-fold conc. ranging from 10^{-3} to 10^{2} times the MIC. The affinity of PBP 1 and 3 was identical for both the Amp^R TF and the Amp^S strain. However, affinity of PBP 5 of the Amp^R TF was decreased 10,000 fold. We conclude that the mechanism of resistance in this TF is alteration in the apparent amount and/or affinity of PBPs.

We compared 6 additional clinical non-typeable Amp^R-β-lactamase-neg. HI (5 from England, 1 USA) to this Amp^S isolate. All 6 revealed differences in their PBP profile; there was strain-to-strain variation in the apparent amount of PBP 3, 4, 5, or 6. We conclude that the mechanism of resistance in these 6 isolates may be altered PBPs.

●1115 THE ROLE OF RESPIRATORY TRACT PROTEASES IN THE INFECTIVITY OF INFLUENZA A VIRUS. Charlotte Morel, Peter F. Wright, Kathryn Edwards. Dept. of Pediatrics, Vanderbilt University, Nashville.

It is recognized that to become fully infectious the influenza hemagglutinin (HA) must be cleaved to HA_1 and HA_2 prior to penetration into cells. It is generally assumed that this cleavage is a function of the cell replication although exogenous proteases, notably trypsin, will cleave virus. We have developed human adenoid fibroblast (HAF) lines which, unlike most other nonembryonic human cells, support the growth of influenza. However, the virus released from HAF cells is not cleaved and only becomes infectious on treatment with protease. The HAF cells have enabled us to approach mechanisms of HA cleavage in the human respiratory tract. The lack of cleavage of influenza by HAF cells and the observation that virus released from an adenoid organ explant was uncleaved suggested that respiratory tract cells might not have intrinsic proteolytic enzymes. Exposure for 2 hours at 37° of HAF grown virus to trypsin (20 µg/ml) or to nasal secretions from children with respiratory tract symptoms restored full infectivity suggesting the presence of exogenous proteases. Polyacrylamide gel electrophoresis of leucine labeled:1) HAF grown virus, 2) HAF grown virus exposed to trypsin and 3) HAF grown virus exposed to nasal secretions confirmed the cleavage in HA_1 and HA_2 with the latter two treatments. To further investigate which proteases present in respiratory secretions might cleave influenza HA, a granulocyte protease preparation and a bacterial IgA protease preparation were utilized: granulocyte proteases cleaved influenza HA while the bacterial IgA protease of an Hemophilus influenzae did not. Further characterization of protease mechanisms in influenza cleavage is needed but an important role for exogenous proteases in influenza pathogenesis is suggested.