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Sarah E. Clark  
Colby College

JaeHee Yun  
Colby College

Justin Guay  
Colby College

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Sarah E. Clark, JaeHee Yun, and Justin Guay

Abstract

Since the development of the first antibiotics in the 1940’s, there has been widespread overuse in both clinical and agricultural applications. Antibiotic resistance has become a significant problem as a result of subsequent dissemination of antibiotics into the environment, and multiple-resistance strains of bacteria are now a major pathogenic threat. In this study eight separate strains of Flavobacterium responsible for recent disease outbreaks in fish hatcheries throughout Maine were collected and analyzed. All eight strains were found to be resistant to high levels of a number of different antibiotics, including those used for aquaculture as well as for human therapeutic applications. Flavobacterium isolates were also shown phenotypically to transfer antibiotic resistance determinants using a conjugation mating system in which Flavobacterium was the donor and Escherichia coli DHA-alpha was the recipient. This experiment suggests that it may be possible for Flavobacterium strains to transfer their multiple antibiotic resistance determinants to human pathogenic bacterial strains. Importantly, none of the hatcheries from which the Flavobacterium isolates were obtained had ever used antibiotics to treat their fish stock. It is possible that there is another selective agent responsible for the development of antibiotic resistance in the absence of antibiotic pressure. Mercury is one possible candidate, as all of the strains tested were resistant to mercuric chloride and it is known that genes encoding antibiotic resistance can be carried on the same mobile genetic elements that encode for mercury resistance. Preliminary data also suggest that the majority of the Flavobacterium isolates contain genes for mercuric ion reduction, which would confirm the mercury resistance genotype.

Conjugation of antibiotic resistance using Flavobacterium donors and E. coli DHA-alpha as the recipient. Relative clinical antibiotic resistance of DHA-alpha and DHA-alpha transconjugants (GH/DHA-alpha, G888/DHA-alpha, PS45/DHA-alpha) to the maximum antibiotic concentration tested is shown. Resistance beyond the maximum concentration tested is represented by bars over 100. The donors in this conjugation mating system, GH, G888, and PS45, are all Flavobacterium isolates.

For the conjugation, one Flavobacterium isolate and DHA-alpha were mixed on BH and incubated at 20°C. Transconjugants were selected first on BH with 8ug/ml CHL (chloramphenicol) at 37°C, then on Hektoen agar with 8ug/ml CHL at 37°C, and finally on BH with 64ug/ml CHL. Isolates from the third BH + CHL plates were used for antibiotic MIC (included at 37°C). Sensitve MIC plates for gram-negative bacteria were used, and manufacturer’s protocol was followed. Results were recorded 4 days after incubation at 20 ºC. Abbreviations: AMP= Ampicillin, A/S= Ampicillin/Sulbactam, GEN= Gentamicin, TET= Tetracycline, CIP= Ciprofloxacin, NOR= Norfloxacin, OFL= Ofloxacin, LOM= Lomefloxacin, COT= Trimethoprim/Sulfamethoxazole, NIT= Nitrofurantoin, FIS= Sulfisoxazole

Clinical antibiotic MIC (minimum inhibitory concentration) values for Flavobacterium isolates. MIC data for all 8 Flavobacterium isolates is shown. Highlighted boxes indicate resistance to the maximum concentration of the antibiotic tested. Blank boxes represent susceptibility.

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References


Mindlin, S.Z., Bass, I.A., Bogdanova, E.S., et al. 1999. Incidence and Characterization of Integrons, which was isolated in the pre-antibiotic era.


