



## TEN-YEAR STUDY OF ANTIBIOTIC RESISTANT BACTERIAL ISOLATES FROM MARINE MAMMALS

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### *I. Introduction*

A. All isolates originated from lesions in 3 species of “distressed” pinnipeds submitted to the Pacific Marine Mammal Center, Laguna Beach, CA from 2004 through 2013: California sea lion (*Zalophus californianus*), harbor seal (*Phoca vitulina*), and northern elephant seal (*Mirounga angustirostris*)

B. Culture swabs were submitted to IDEXX Laboratories, Los Angeles, CA for culture and sensitivity testing. There were 7 classes of antibiotics:

1. Penicillins: amoxicillin, Clavamox, piperacillin
2. Cephalosporins: ceftioxime, ceftiofur, cephalexin
3. Aminoglycosides: amikacin, gentamicin, tobramycin
4. Fluoroquinolone: ciprofloxacin, enrofloxacin
5. Tetracyclines: tetracycline
6. Phenicol: chloramphenicol
7. Folate pathway inhibitor: trimethoprim/sulfa

C. Definitions<sup>1</sup>

1. Non-susceptible isolate: reported as “resistant” or “intermediate”
2. Multidrug Resistant (MDR): non-susceptibility to at least one agent in 3 or more antibiotic classes
3. Extensive Drug Resistant (XDR): non-susceptibility to at least one agent in 5 or more antibiotic classes

D. Bacterial species were grouped in two ways

1. Gram-negative vs. gram-positive
2. “Environmental” (E) (species likely to be acquired from the human-dominated microbiological environment) vs. “Marine” (M) (species indigenous to marine mammals and their natural food chain)

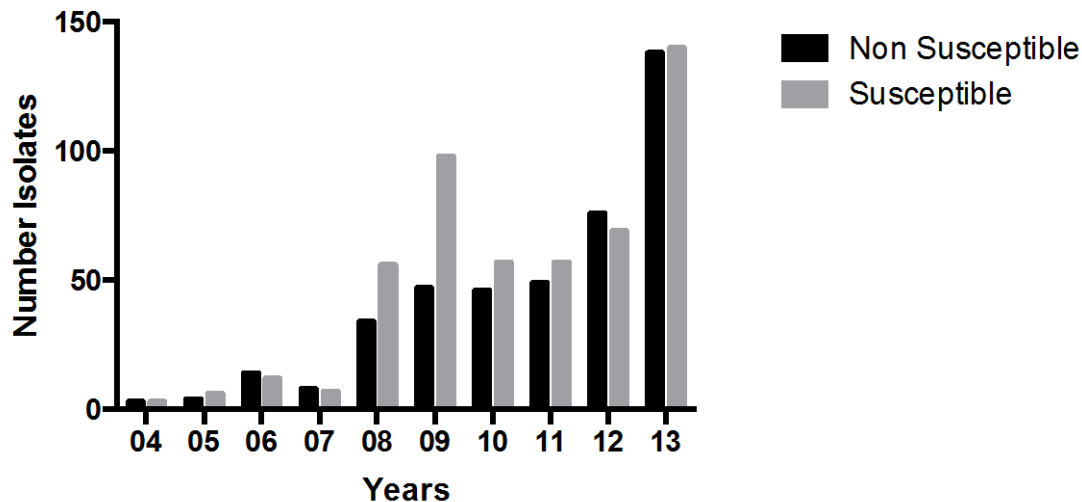
E. Statistics

1. Proportions were expressed as percentages
2. Comparison of categorical variables employed the chi-square tests for independence and for trend (Prism<sup>®</sup> GraphPad, Inc., La Jolla, CA)

## II. Overall Findings

- A. Total number of isolates: 925
  1. E Gram-negative: 74%
  2. E Gram-positive: 16%
  3. M Gram-negative: 4%
  4. M Gram-positive: 6%
- B. Non-susceptible: 45% of total isolates
  1. MDR: 60% of all non-susceptible; 27% of total isolates
  2. XDR: 40% of all non-susceptible; 18% of total isolates
- C. 10-year data
  1. All non-susceptible vs. susceptible isolates  
 Independence:  $X^2=18.2$ ;  $df=9$ ;  $p=0.03$   
 Trend:  $X^2=5.8$ ;  $df=1$ ;  $p=0.016$

### 10-Year Data All isolates



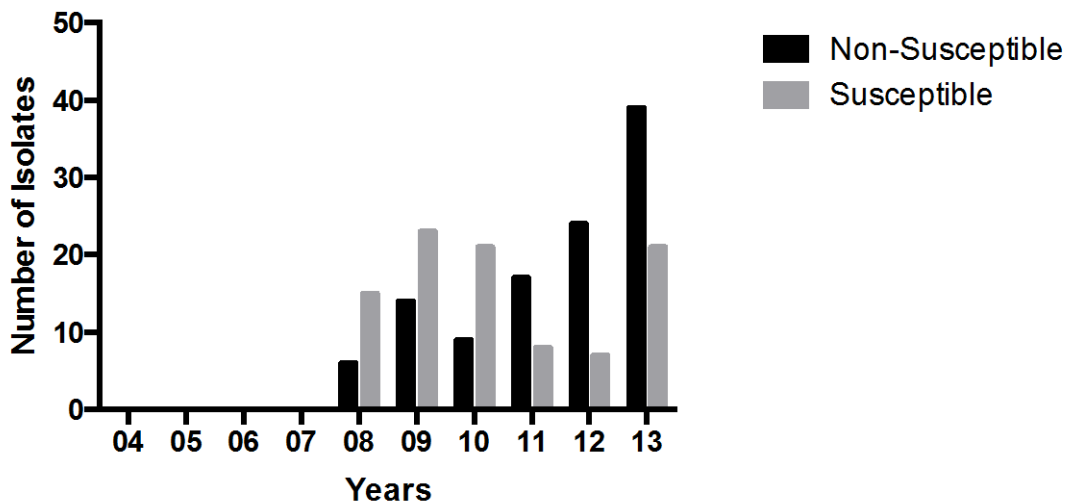
2. E Gram-negative isolates: non-susceptible vs. susceptible  
 Independence:  $X^2=53.2$ ;  $df=1$ ,  $p<0.0001$   
 Trend:  $X^2=31$ ;  $df=1$ ;  $p<0.0001$
3. For the other bacterial groups, the small numbers (or zero) in some categories rendered chi-square analysis invalid.
4. There was a significant association between the year and the prevalence of non-susceptible isolates. Furthermore, there was a significant positive trend. The overall data were dominated by the Gram-negative group (comprised of the Enterobacteriaceae) which exhibited both the association and trend to a more marked degree.

### III. Individual bacterial species

#### A. *Escherichia coli*

1. 33% of all E Gram-negative isolates.
2. 53% were MDR/XDR which accounts for 31% of all E Gram-negative non-susceptible isolates.
3. 10-year data (due to low numbers of isolates in the early years, valid chi-square results were possible only for 2008-2013)
  - Independence:  $X^2=28$ ;  $df=9$ ;  $p=0.001$
  - Trend:  $X^2=19.5$ ;  $df=1$ ;  $p<0.0001$
4. There is a significant association between the proportion of non-susceptible isolates and years, and a significant positive trend.

#### 6-Year Data *E. coli*



#### B. *Enterococcus* sp.

1. 50% of total E Gram-positive isolates.
2. 25% were MDR/XDR
3. Due to low (or zero) numbers in several categories, chi-square is invalid.

#### C. Environmental vs. Marine isolates

1. Non-susceptible isolates in the Environmental group: 50%  
Non-susceptible isolates in the Marine group: 4%
2. Indigenous bacteria displayed very low prevalence of non-susceptibility.
3. *Shewanella* sp. and *Archanobacterium phocae* accounted for 73% of the M Gram-negative isolates.
4. *Streptococcus phocae* accounted for 100% of the M Gram-positive isolates.

#### **IV. Host species**

A. Percent non-susceptible isolates from each host:

Sea lion: 45%

Elephant seal: 45%

Harbor seal: 48%

B.  $X^2=0.24$ ;  $df=2$ ;  $p=0.89$ . No association between host species & bacterial non-susceptibility.

#### **V. Anatomical location of lesions (all isolates)**

A. Percentages

Abdomen: 48%

Thorax: 43%

Head: 45%

Skin: 45%

CNS: 46%

B.  $X^2=1.1$ ;  $df=4$ ;  $p=0.89$ . No association between anatomical location of lesions and bacterial non-susceptibility.

#### **VI. Conclusions**

The data revealed a clear trend toward increasing antibiotic resistance in the isolates from lesions in distressed marine pinnipeds. This trend was largely driven by the preponderance of non-susceptible bacteria derived from the human-dominated environment: the Enterobacteriaceae, especially *E. coli*. It would appear that these resistant bacteria may out compete sea lion/seal-endogenous species during the development of infection.

#### **Reference:**

1. Magiorakos A-P, Srinivasab A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 18:268-281. 2012