Temporal Evaluation of Antibiotic Resistance from Common Bottlenose Dolphin (*Tursiops truncatus*), a Sentinel Species

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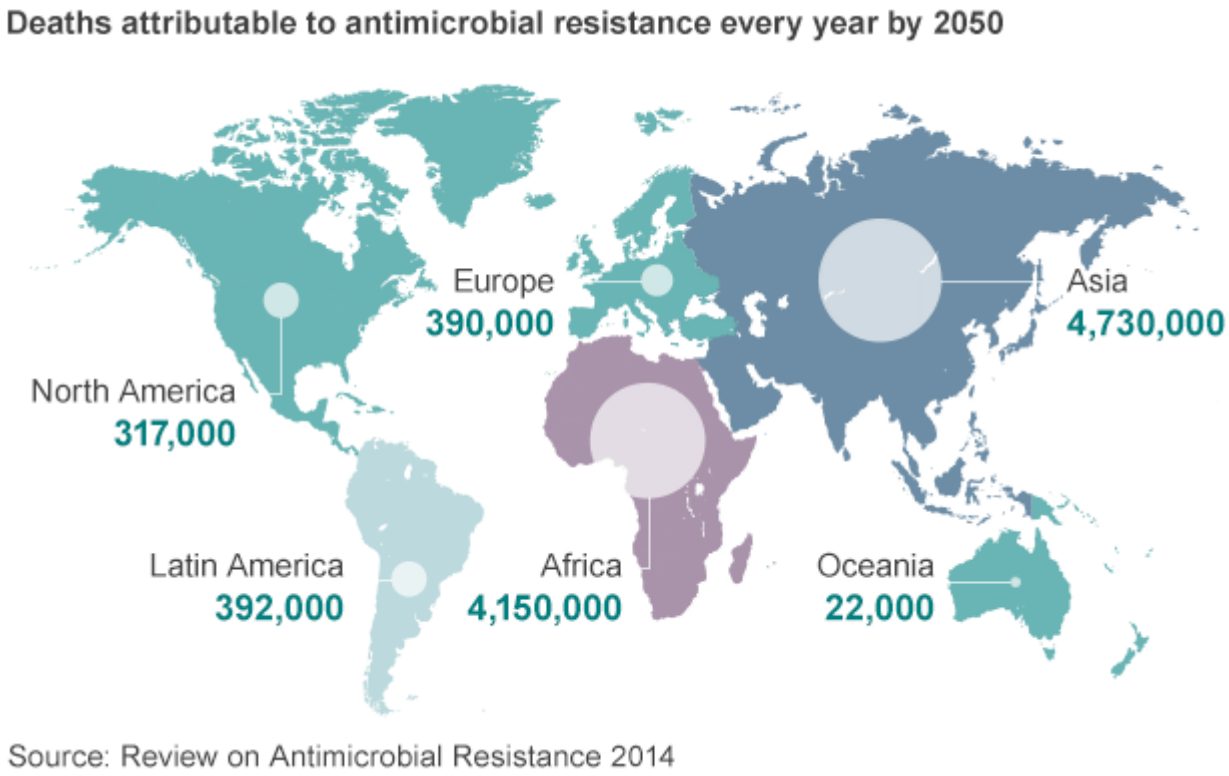
A Global Concern

- Resistance is an emerging health threat worldwide
  - > 70% of hospital-associated pathogens are resistant to at least one antibiotic commonly used to treat it leading to increased mortality
  - The marine environment serves as a potential reservoir for antibiotic resistance



Antibiotic Resistance in Wild Marine Species

- Resistant Bacteria cultured from:
  - Demeral & pelagic fish (Miranda and Zemelman, 2007)
  - Multiple Shark species (Blackburn, 2003)
  - Pinnipeds *(Johnson et al., 1998)*
  - Bottlenose dolphins (Stewart et al., 2014; Schaefer et al., 2009)

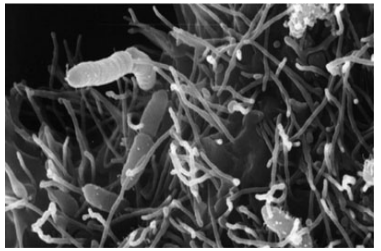
Microbial Identification and Sensitivity

Blowhole

Fecal

Gastric

Isolates



• Swabs from the blowhole 394 (53.7%) , gastric fluid 140 (19.1%) and fecal 199 (27.2%) were collected (n =733)

Organism	2003-2007 n (%)	2010-2015 n (%)	Total n (%)
Acinetobacter baumannii	25 (5.7)	10 (3.4)	35 (4.8)
Aeromonas hydrophila	76 (17.5)	53 (17.8)	129 (17.6)
Escherichia coli	23 (5.3)*	37 (12.4)*	60 (8.2)
Edwardsiella tarda	20 (4.6)*	30 (10.1)*	50 (6.8)
Klebsiella pneumoniae	7 (1.6)	6 (2.0)	13 (1.8)
Pseudomonas aeruginosa	13 (3.0)	6 (2.0)	19 (2.6)
Staphylococcus aureus	11 (2.5)*	26 (8.7)*	37 (5.0)
Vibrio alginolyticus	28 (6.4)	10 (3.4)	38 (5.2)
Other Species	232 (55.3)*	120 (40.3)*	352 (48.0)

* Significantly different

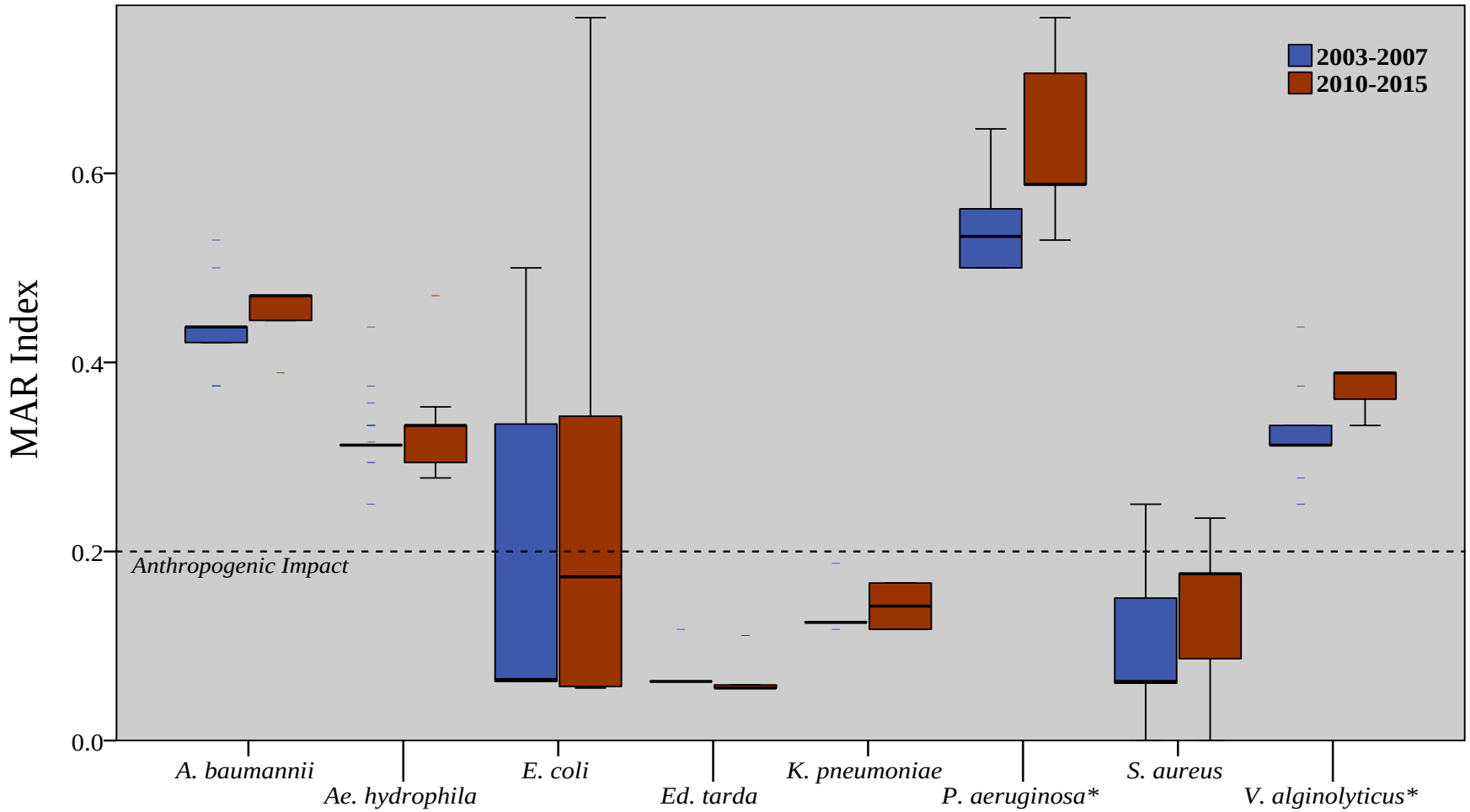
Multiple Antibiotic Resistance (MAR) index

$$\frac{\#\ Antibiotics\ with\ Resistance}{Total\ \#\ Antibiotics\ Screened} = MAR\ Index$$

- A value of > 0.20 is interpreted as an indicator of anthropogenic pollution (Paul et al. 1997; Vivekanandhan et al. 2002; Chitanand et al. 2010).

- In aquatic ecosystems, the MAR index has been used as an indicator of anthropogenic pollution (Krumperman 1983; Kaspar et al.1990; Parveen et al. 1997; Harwood et al. 2000; Kelsey et al. 2003; Webster et al.2004; Sayah et al. 2005; Wallace et al. 2013; Watkinson et al. 2007).

MAR Index Between Sampling Periods



Individual Antibiotics

Antibiotic	AM	AK	AU	C	CE	CF	CH	CI	EN	ER	FU	GE	PI	TE	ST
Acinetobacter baumannii															
2003-2007	80.1	0.3	67.1	3.2	3.2	40.0	17.4	1.5	1.5	87.4	18.9	0.3	66.0	12.9	6.7
2010-2015	69.4	0	52.0	6.7*	8.3*	35.0	12.8	2.2*	1.7	92.2*	13.9	1.7*	50.0	8.9	8.9*
Aeromonas hydrophila															
2003-2007	100	0	100	0	0	25.0	1.3	0	0	0	1.3	0	98.7	0	0
2010-2015	98.1	0	90.4	0	0	59.5*	1.9	0	0	0	1.9	0	100*	0	0
Escherichia coli															
2003-2007	40.0	0	30.0	0	5.0	100	25.0	0	0	0	25.8	0	15.0	10.0	15.0
2010-2015	48.0	0	48.0*	20.0*	16.0*	35.0	20.0	12.0*	8.0	0	24.0	4.0*	32.0*	24.0*	28.0*
Edwardsiella tarda															
2003-2007	0	0	0	0	0	0	0	0	0	100	5.0	0	0	0	0
2010-2015	0	0	0	0	0	0	3.4	0	0	96.6	0.0	0	3.4	0	0
Klebsiella pneumoniae															
2003-2007	100	0	0	0	0	0	0	0	0	100	0	0	14.3	0	0
2010-2015	100	0	0	0	0	0	0	0	0	100	16.7*	0	0	0	0
Pseudomonas aeruginosa															
2003-2007	100	0	100	38.5	7.7	0	0	0	0	0	0	0	7.7	0	0
2010-2015	100	0	83.3	83.3*	16.7*	0	0	16.7*	16.7	0	0	33.3*	16.7*	0	0
Vibrio alginolyticus															
2003-2007	100	3.6	0	0	0	0	3.6	0	0	96.4	3.6	0	96.4	0	0
2010-2015	100	0	0	0	10.0*	0	0	0	0	100*	0	0	100*	10	10
Staphylococcus aureus															
2003-2007	63.6	0	0	0	36.4	0	0	9.1	9.1	9.1	0	0	18.2	0	9.1
2010-2015	65.4	0	0	0	34.6	0	0	0	0	53.8*	0	0	3.8	7.7	3.8

* Statistically Significant different from 2003-2007 sampling period by chi square ($p < 0.05$).

AM = ampicillin, AK = amikacin, AU = amoxicillin , C = ceftifur ,CE = cefotaxime, CF = ceftazidime, CH = chloramphenicol, CI = ciprofloxacin, EN = enrofloxacin, ER = erythomycin, FU = furadantin, GE = gentamicin, MA = marbofloxacin, PI = piperacillin, TE tetracycline, ST = sulfamethoxazole/trimethoprim

Results

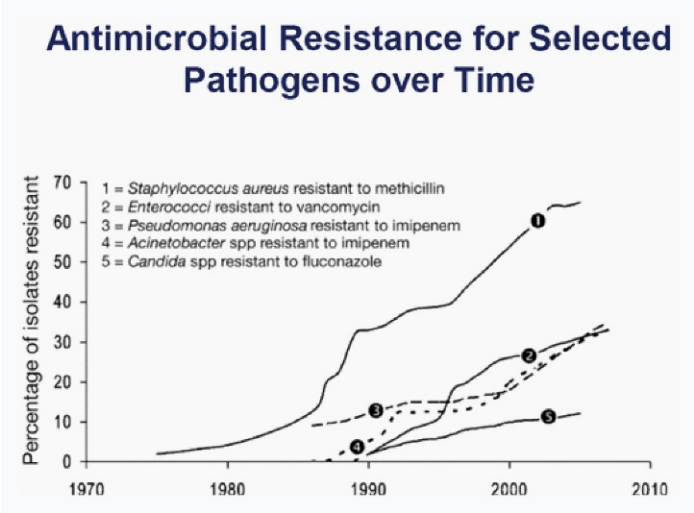
- Resistance to 3rd generation Cephalosporins increased among *V. alginolyticus*, *P. aeruginosa* and *E. coli*

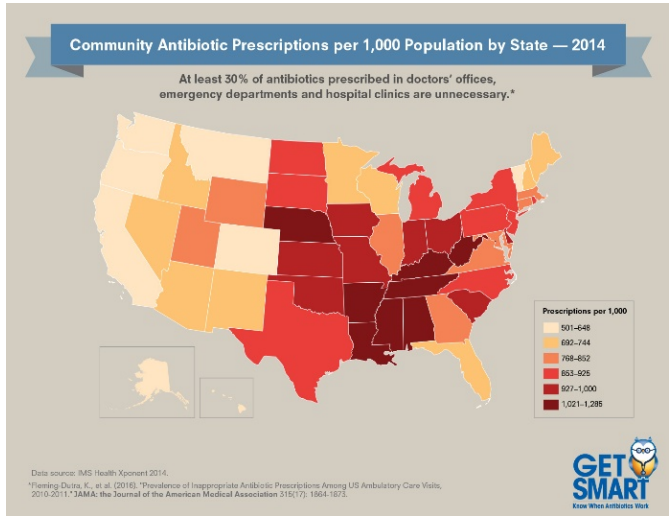
- Resistance to Fluoroquinolones increased among *A. baumannii*, *E. coli*, and *P. aeruginosa*
  - Parallels increase in resistance observed among same taxa isolated from pinnipeds (Wallace et al. 2013)

- Resistance to Ciprofloxacin more than doubled among *E. coli*
  - *Cipro resistance among human isolates increased x 8 between 1991-2006 (Medalla et al. 2013)*

Antibiotic Usage and Resistance

- Significant relationships between resistance and antibiotic usage have been observed among human isolates





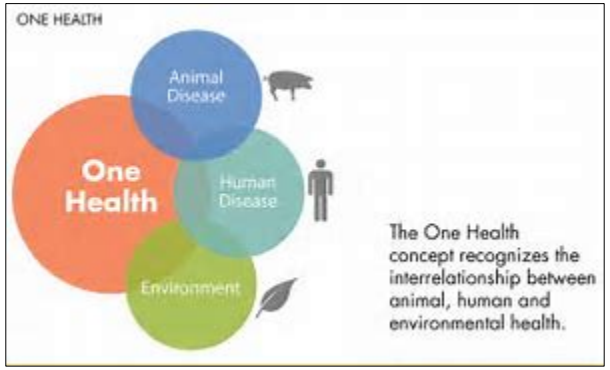
- Current results demonstrate temporal increases that parallel human antibiotic consumption of:
  - Cephalosporins (Van Boeckel et al. 2014)
  - Fluoroquinolones (Linder et al. 2005; May et al. 2014)

Summary

- Multiple MAR indices of some pathogens reflect anthropogenic impacts along the IRL

- Changes in resistance parallel trends in the usage and resistance of antibiotics in human populations

- Changes in patterns of resistance among pathogens in wildlife represent potential human health risk



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