

Submitted ASM  
(CAAE decision 6/27/03)

**Title:** Association of nasal carriage of methicillin resistant and multiple antibiotic resistant coagulase negative staphylococci species with deficiency of alpha melanocyte stimulating hormone in Chronic Fatigue Syndrome: implications for expanded treatment options

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**Background:** Deficiency of  $\alpha$  melanocyte stimulating hormone (MSH), a hypothalamic neuroimmune modulating, anti-inflammatory factor, is a marker for chronic, biotoxin-associated illness (CBAI). CBAI is evidenced by a pro-inflammatory cytokine response to biotoxins, with multiple symptoms affecting multiple organ systems, especially fatigue, chronic pain and executive cognitive dysfunction. CBAI patients often meet the broad CDC definition for Chronic Fatigue Syndrome (CFS); they have acquired MSH deficiency following exposure to toxigenic organisms, including dinoflagellates (Possible Estuarine Associated Syndrome, ciguatera, *Chattonella*), fungi (Sick Building Syndrome), spirochetes (Post-Lyme syndrome), apicomplexans (*Babesia* and *Sarcocystis*), cyanobacteria (*Microcystis*, *Lyngbya* and *Cylindrospermopsis*) and brown recluse spiders (*Loxosceles*). These patients show considerable reduction in symptoms when treated with cholestyramine (CSM), an orally administered, non-absorbable, anion-binding resin. Patients with less robust symptomatic response to CSM frequently have coagulase negative staphylococci (CoNS) colonizing deep nasal tissue, especially those with methicillin resistance (MRCoNS) and CoNS with multiple antibiotic resistances (MAR-CoNS). We obtained 571 cultures from 512 patients, including 113 asymptomatic and 399 symptomatic due to CBAI. Deficiencies in MSH were again noted to be associated with symptoms. Treatment responses showed differences based on presence of CoNS.

MRCoNS were associated with the poorest response to antibiotic treatment following CSM and the greatest risk of relapse of symptoms coincident by reacquisition of CoNS, complicated by additional antibiotic resistance. MSH deficient CBAI patients colonized with CoNS with antibiotic-sensitive or single-antibiotic resistance CoNS responded to treatment equally as CBAI patients without CoNS.

**Methods:** All patients provided informed consent for culture and treatment at a single clinical site. Aerobic nasal cultures taken deep to the middle turbinates were cultured for isolation and speciation of CoNS, using the API-STAPH system. Sensitivities were determined by Kirby-Bauer agar diffusion, in accordance with NCCLS protocols. Control patients had <2 of 18 possible symptoms, consistent with previous CBAI control groups. Patients with symptoms and exposure were treated with 9 grams CSM, four times a day, for 30 days. Patients with a positive CoNS culture and persistent symptoms

were treated for a second 30-day interval with CSM taken two times a day and a triple antibiotic regimen of muciprocin applied topically on nasal mucosa three times a day, rifampin 600 mg taken at one dose daily and sulfamethoxazole-trimethoprim DS (or doxycycline 100 mg twice a day in sulfa allergic patients). Symptoms were recorded and the patients monitored for relapse. Follow-up symptoms of patients returning to the site were recorded.

**Results:** There were no dropouts from medication side effects, though sulfa allergy was noted in 5% of antibiotic-treated patients. Statistically significant differences included: MSH deficiency was not seen in 97% of controls (average 34.4 pg/ml, normal 26-50). MSH deficiency was found in >93% of symptomatic patients. Cultures were negative in 64% of 116 controls and 18% of 455 cases. Of the CoNS positive cultures in control patients, none had more than 1 antibiotic resistance, with similar distribution of resistance to those in 71 cases with 1 resistance. MAR-CoNS species were found in 302 symptomatic patients, including 192 with methicillin resistance. The predominant species isolated was *S. epidermidis* (92%). Symptom reduction following CSM alone in symptomatic patients with no CoNS was from 10.1 to 1.2, equal to controls; presence of CoNS with one antibiotic resistance was 10.0 to 1.8, each of these groups without relapse following CSM; presence of >1 resistance, not methicillin, was 14.1 to 4.9, with 2.2 symptoms after antibiotics; and MRCoNS 16.3 to 9.2, with reduction to 4.8 following antibiotics. Test of cure was obtained in 69 MRCoNS patients and 34 CoNS patients. 17% of antibiotic-treated MRCoNS patients and 6% of CoNS patients relapsed with repeated positive CoNS cultures within 2 months following antibiotic therapy. There were no differences in CoNS antibiotic resistance profiles between primary CBAI diagnoses. The average number of antibiotic resistances was 2.4 and 4.4 in the CoNS and MRCoNS groups, respectively. 5% of the MR-CoNS isolates were resistant to 4 or more antibiotic classes. 60% of the MRCoNS isolates were resistant to 4 or more antibiotic classes; 41% resistant to 5 or more; 7% resistant to 6 antibiotic classes and 4 % resistant to 7 antibiotic classes, including gentamicin. No isolates were vancomycin resistant.

**Conclusion:** This study suggests that multiple-antibiotic resistant CoNS, especially MRCoNS, are potential pathogens in CBAI/CFS patients with MSH deficiency. Presence of MRCoNS is associated with multiple persistent symptoms and a high rate of relapse following therapy. The observed development of antibiotic resistance following antibiotic therapy for CoNS and the existence of "new" antibiotic resistances in these putative normal flora, support the concept that CoNS is changing. Presence of methicillin resistant *S. aureus* in the community, with possible plasmid exchange of resistance factors and widespread use of broad-spectrum antibiotics possibly could be forces driving selection for MAR-CoNS. Antibiotics are unlikely to provide long-term elimination of nasal carriage of CoNS. Additional treatment modalities, including Staph vaccines, topical bismuth thiol compounds and MSH replacement, show promise for future therapy in patients with MSH deficiency and symptoms consistent with CFS.

### Age/Sex By Cohort

	Control		Sympt + Culture Neg		Symt <2 resist		Not Methicillin		Methicillin Resistant		
		#	%	#	%	#	%	#	%	#	%
BM	0-20	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	21-35	1	0.9%	0	0.0%	0	0.0%	0	0.0%	1	0.5%
	36-50	1	0.9%	0	0.0%	1	1.4%	0	0.0%	2	1.0%
	51-65	0	0.0%	1	1.2%	1	1.4%	0	0.0%	1	0.5%
	>65	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
WM	0-20	7	6.2%	0	0.0%	1	1.4%	0	0.0%	2	1.0%
	21-35	10	8.8%	4	4.9%	1	1.4%	8	7.2%	8	4.2%
	36-50	14	12.4%	7	8.5%	8	11.3%	17	15.4%	25	13.0%
	51-65	18	15.9%	9	11.0%	8	11.3%	17	15.4%	27	14.1%
	>65	7	6.2%	5	6.1%	0	0.0%	3	2.7%	8	4.2%
BF	0-20	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
	21-35	1	0.9%	2	2.4%	1	1.4%	1	0.9%	0	0.0%
	36-50	2	0.2%	2	2.4%	2	2.8%	1	0.9%	1	0.5%
	51-65	0	0.0%	0	0.0%	1	1.4%	1	0.9%	2	1.0%
	>65	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
WF	0-20	6	5.3%	2	2.4%	1	1.4%	6	5.4%	10	5.2%
	21-35	13	6.7%	9	11.0%	2	2.8%	9	8.2%	16	8.3%
	36-50	19	16.8%	22	26.8%	19	23.2%	24	21.8%	47	24.5%
	51-65	6	5.3%	17	20.7%	17	20.7%	19	17.3%	36	18.6%
	>65	8	7.1%	2	2.4%	5	6.1%	4	3.6%	5	2.6%
N=		113		82		71		110		192	

Asian F 36-50 (2)

## MSH Levels by Cohort

MSH

	Control		Sympt + Cult Neg		Sympt <2 Resis		Not Methicillin		Methicillin	
	N	%	N	%	N	%	N	%	N	%
<15	1	3	36	51	46	71	63	65	104	63
16-25	6	20	28	39	15	23	30	31	49	30
26-35	9	30	5	7	2	3	3	3	12	7
36-45	8	26	2	3	2	3	1	1	1	1
46-55	3	10	0	0	0	0	0	0	0	0
>55	3	10	0	0	0	0	0	0	0	0
<b>Total</b>	<b>30</b>		<b>71</b>		<b>65</b>		<b>97</b>		<b>166</b>	

## Characteristics of Positive Culture in Control

Controls    All Cases

N=	116	455
Neg Cult	64.0%	18.0%
PCN Resis	18.0%	8.0%
Macro Resis	5.0%	1.3%
No Resis	13.0%	5.0%
2 or More Resis	0.0%	66.0%
Methi Resis	0.0%	42.0%

Control ~~and~~ parallels + <2 resis

	Controls	<2 ABX Resis
No resis	36.0%	37.0%
Pen only	50.0%	52.0%
Macro only	13.0%	8.0%
TCN only	0.0%	1.4%
Sulfa only	0.0%	1.4%

### Chronic Biotoxin Associated Illness Diagnoses

	Total in Cohort		Sympt + Culture Neg		Sympt <2 Resis		Not Methicillin		Methicillin	
	Total	Percentage	Total	Percentage	Total	Percentage	Total	Percentage	Total	Percentage
Post Lyme Syndrome	136	27.9	18	18.9	13	18.6	31	27.0	74	35.6
Sick Building Syndrome	244	50.0	53	55.8	38	54.5	61	53.0	92	44.2
Chronic Fatigue Syndrome	40	8.2	6	6.3	6	8.6	11	9.6	17	8.2
Ciguatera	22	4.5	4	4.2	8	11.4	5	4.3	5	2.4
Pfiesteria	7	2.4	1	1.0	1	1.4	2	1.7	3	1.4
Coagulose Negative Staph only	28	5.7	2	2.1	4	5.7	5	4.3	17	8.2
N	477		84		70		115		208	

### Symptoms (out of 24)

	Before RX CSM	After Rx CSM	After ABX
+ Symptoms Cult Neg	10.1	1.2	N/A
+ Symptom <2 Resist	10.0	1.8	N/A
Not methicillin resistant	14.1	4.9	2.2
Methicillin resistant	16.3	9.2	4.8
Control	1.2	N/A	N/A

## Multiple Cultures Positive in 1 Patient

	N	# Cultures	Multiple	% Multiple
Control	113	116	3	2.6
Symptom + Cult Neg	80	82	2	2.4
Symptom <2	64	71	7	9.8
Not Methicillin	92	110	18	16.4
Methicillin	163	192	29	15.1

### Culture Results for all Groups

	N	%
Control Cult	36/116	31
Symptomatic, Entire Group	373/455	82
Symptoms + <2 resist	71/373	19
Not Methicillin	110/373	25.9
Methicillin	192/373	51.5

### Multiple Resistances

	N	% of Total
Not Methicillin	110	36.4
Methicillin	192	63.6



### Distribution of ABX Resistant MAR-CNS

N Resist by ABX Class	Distribution of ABX resis MAR-CNS	N Culture +
2	Macro + PCN	57
2	PCN + Ceph	2
2	PCN + TCN	4
2	PCN + Quin	4
2	PCN + Sulfa	6
2	Macro + PCN	8
2	Sulfa + Quin	2
3	PCN Quin Sulfa	4
3	PCN Quin Macrolide	7
3	PCN Macro TCN	9
3	PCN Sulfa TCN	1
4	PCN Macro TCN Sulfa	1
4	PCN Macro Sulfa Rifampin	2
5	PCN Macro Quin TCN Sulfa	2
6	PCN Macro Quin TCN Sulfa Gent	1
	N=	110

Average # Resistances = 2.2

## Distribution of Methicillin Resistant

Controls	None
+ Sx negative culture	None
+ Sx + culture <2 resistances	None
+ Sx methicillin resistant	192

# Resistances by ABX Class	Distribution of Methicillin Resistances	
2	Meth + PCN	27
3	Meth PCN Macro	32
3	Meth PCN Quin	11
3	Meth PCN Ceph	5
3	Meth PCN TCN	1
4	Meth PCN Macro Cephal	22
4	Meth PCN Macro Quin	17
4	Meth PCN Macro Sulfa	4
4	Meth PCN Macro TCN	5
4	Meth PCN Macro Rifampin	1
4	Meth PCN Quin Ceph	6
4	Meth PCN Quin TCN	1
4	Meth PCN Ceph TCN	2
5	Meth PCN Macro Quin TCN	3
5	Meth PCN Macro Cepha TCN	7
5	Meth PCN Macro Quin Ceph	17
5	Meth PCN Macro Quin Sulfa	4
5	Meth PCN Macro Quin Rifampin	3
5	Meth PCN Macro Rifampin Sulfa	2
5	Meth PCN Quin TCN Sulfa	1
5	Meth PCN Ceph TCN Sulfa	1
6	Meth PCN Macro Ceph TCN Sulfa	3
6	Meth PCN Macro Quin Ceph TCN	10
6	Meth PCN Macro Quin Ceph Rifampin	1
7	Meth PCN Macro Quin Ceph TCN Sulfa	4
7	Meth PCN Macro Quin Ceph Sulfa Gent	2

Average # resistances	4.4
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Follow up ABX RX

	Test of Cure	# Relapse	% Relapse	# Relapse With Resistant Org	% Relapse With Resistant Org
Methicillin	69	12	17	15/17	88
Not Methicillin	34	2	6	5/6	83