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Sponsor/company:	sanofi-aventis	ClinialTrials.gov Identifier:	NCT00424905
		Study Code:	PM_L_0199
Generic drug name:	Bacillus clausii multi-antibioresistant spores	Date:	28-nov-2008

Title of the study:	Evaluation of the effects of Enterogermina, 2 billion *Bacillus clausii* spores, on the intestinal flora of children antibiotic treated for bacterial upper respiratory tract infections: open, pilot study (study code: PM_L_0199)
Investigator:	Dr Sergio Amarri Division of Paediatrics Santa Maria Nuova Hospital Viale Risorgimento, 80 42100 Reggio Emilia
Microbiology Laboratory	Prof Lorenzo Morelli Advanced Analytical Technologies (AAT) srl Via Emilia Parmense, 84 29100 Piacenza (Italy)
Study center:	1 center in Italy
Publications (reference):	Not applicable
Study period: Date first patient enrolled: 15-Dec-2006 Date last patient completed: 28-Dec-2007	Phase of development: IV
Objectives:	<u>Primary</u> To assess the effects of *Bacillus clausii* (Enterogermina®) on fecal microbial flora (using PCR-DGGE method) in antibiotic-treated children with complicated acute otitis media or beta-hemolytic streptococcal pharyngo-tonsillitis. <u>Secondary</u> To assess the effects of *Bacillus clausii* (Enterogermina®) on GI symptoms and signs in antibiotic-treated children with complicated acute otitis media or beta-hemolytic streptococcal pharyngo-tonsillitis; To evaluate the presence of *Bacillus clausii* in fecal samples; To assess the safety and tolerability of *Bacillus clausii* (Enterogermina®) through physical examination and treatment emergent adverse events monitoring.
Methodology:	A single-center, open-label, national, controlled, parallel group pilot study in antibiotic-treated children with complicated acute otitis media or beta-hemolytic streptococcal pharyngo-tonsillitis

Number of patients:	Planned: 50 children (25 treated with Enterogermina® + antibiotic; 25 treated with antibiotic only)	Randomized: 60	Treated: 55
Evaluated:	Efficacy: The ITT population (40 patients) was considered for efficacy analyses. A supportive efficacy analysis of primary variables was performed also on the PP population (30 patients).	Safety: Safety parameters were evaluated in the safety population (55 patients).	
Diagnosis and criteria for inclusion:	• Children of either sex, aged ≥ 1 and < 6 years; • Patients with persistency of symptoms and requiring antibiotics for bacterial upper respiratory tract infections, i.e. complicated acute otitis media or beta-hemolytic streptococcal pharyngo-tonsillitis; • Outpatients or patients attending an emergency room for both diagnoses, and inpatients in case of complicated acute otitis media; • Written informed consent from both parents		
Investigational product: Dose: Administration:	Enterogermina®; Amoxicillin Enterogermina®: Vials containing 2×10^9 spores of antibiotic-resistant *Bacillus clausii*. 2 Enterogermina® vials/day Amoxicillin: 50 mg/kg/day divided in 3 daily doses (every 8 hours) Both via oral route		
Duration of treatment: Enterogermina®: 12 to 17 days (i.e. during the 5 to 10-days antibiotic treatment and for 7 days thereafter) Amoxicillin: 5 to 10 days		Duration of observation: Ranging from 13 to 18 (+ max. 2) days	
Reference therapy:	No treatment with Enterogermina®		
Criteria for evaluation:			
Efficacy:	a) Primary end-point Changes of microbial composition of fecal bacterial flora by PCR-DGGE analysis (differences in bacterial groups between samples obtained at baseline and follow-up); b) Secondary end-points • Changes from baseline of body weight; • Assessment of abdominal symptoms such as: abdominal discomfort or pain, abdominal bloating, straining, stool frequency and consistency (measured by questionnaires and/or rating scales); • Presence of *Bacillus clausii* spores in feces; Number and rate of patients with GI symptoms (including diarrhea) and time to first development of symptoms		

Safety:	Safety assessments consisted of monitoring and recording all treatment emergent adverse events (TEAEs) and serious adverse events (SAEs). The safety endpoint was the incidence and severity of TEAEs occurred during the study treatment.
Statistical methods:	Sample size justification was not applicable, due to the pilot nature of the study, which was aimed at obtaining a preliminary estimation of the effect of *Bacillus clausii* on GI microflora. The ITT population was considered for efficacy analyses. A supportive efficacy analysis of primary variables was performed also on the PP population. Safety parameters were evaluated in the safety population. The statistical analyses on the primary efficacy variable were performed on similarity coefficient (%) on the basis of the three different algorithms: Dice, Pearson and Jaccard. Two statistical approaches were considered. First, for Dice and Pearson algorithms, each patient was classified as responder if the similarity coefficient was greater or equal 80(%),otherwise as a non-responder. According both the available lliterature and the Microbiologists using this new sophisticated technic, similarity was considered maintained if results ranged between 80 and 100% . It means, that in case of values upper 80%, similarity is maintained; if results are lower,there is an impairment of the microbiota. For Jaccard algorithm the classification was not applicable, as literature did not support a categorical approach. Using the chi-square test and the odds ratio with the relevant 95% confidence interval along as p-value by using a logistic regression model was reported compared the proportion of responders. Interaction between age (age was classified as ≤ 2 years and >2 years) and treatment (at 5% level, two-sided) was tested. Median Similarity Coefficient (MSC) and Inter Quartile Range (IQR) were calculated for each group and algorithm. Similarity coefficient (%) was also analyzed, for each algorithm, as continuous variable by means of Analysis of Variance (ANOVA). In case of severe departure from the ANOVA assumptions (the distribution should be right-skewed) a supportive non-parametric analysis would have been considered. Using a non-parametric ANOVA model would have compared the two treatments groups. In this case to assess the difference between treatment groups, the difference in the medians would have been estimated (using Hodges-Lehmann method). The relative 95% confidence interval along with the p-value would have been reported. Within the analysis of secondary efficacy variables, changes from baseline of body weight were analyzed by ANCOVA (Analysis of Covariance) with baseline values as linear covariate and treatment as main effect. Interaction between treatment and baseline value was tested. The evaluation of daily changes of abdominal symptoms and other GI effects within group was assessed by means of Friedman Test while between groups by Mann−Whitney−Wilcoxon test. A descriptive analysis of *Bacillus clausii* spores was performed to highlight characteristics between baseline and final feces samples. The incidence of TEAEs was calculated as ratio between the number of patients with one or more TEAE and the number of patients included in the safety population. The relative risk of Enterogermina® vs. untreated arm of TEAEs and the 95% confidence intervals were calculated.

Summary:

The demographic characteristics of the two treatment groups for all randomized patients were similar with the exception of gender distribution. The percentage of male patients was higher (63.3%) in the Amoxicillin plus Enterogermina® group than in the Amoxicillin group (35.7%), the difference in the proportion was statistically significant. The mean (SD) patient's age was approximately 40 (18.88) months in the Amoxicillin plus Enterogermina® arm and 42.1 (18.94) months in the Amoxicillin arm. More than eighty percent of patients were Caucasian.

Demographic characteristics - All randomized patients

			A + E	A	P-value
No. of randomized patients		n (%)	30 (100.0%)	28 (100.0%)	
Sex	Male	n (%)	19 (63.3%)	10 (35.7%)	0.036*
	Female		11 (36.7%)	18 (64.3%)	
Age [months]		N	30	28	0.673°
		Mean	40.0	42.1	
		Std	18.88	18.94	
		Median	39.0	40.5	
		Q1 ; Q3	26.0 ; 51.0	28.5 ; 59.0	
		Min ; Max	12.0 ; 74.0	11.0 ; 75.0	
Race	Caucasian	n (%)	29 (96.7%)	23 (82.1%)	0.157*
	Asian		0 (0.0%)	1 (3.6%)	
	Black		0 (0.0%)	1 (3.6%)	
	Hispanic		1 (3.3%)	0 (0.0%)	
	Other		0 (0.0%)	3 (10.7%)	

A + E = Amoxicillin + Enterogermina®; A=Amoxicillin
° P-value based on t-test.
* P-value based on Chi-square test.
Source data: patient data listing 14.2.4.1

Primary diagnosis on all randomized patients was complicated acute otitis media on both treatment groups (63.3% in the Amoxicillin plus Enterogermina® group, 67.9% in the Amoxicillin group).

Overall, treatment compliance of Amoxicillin was good in both treatment groups with the mean percent compliance greater than 92%. Only one patient reported a compliance less than 80% in the Amoxicillin plus Enterogermina® arm. There was a good treatment compliance of Enterogermina® with a mean (SD) percent value of 94.9 (11.37)%. Only one patient reported compliance less than 80%.

Efficacy results:

Primary efficacy variable(s)

The primary analysis was the change from baseline of the microbial composition of fecal bacterial flora by DGGE analysis.

No significant change in intestinal microflora was observed in any group after antibiotic treatment. No statistically significant differences regarding the incidence of treatment success were found between the two treatment groups for the three algorithms Dice, Pearson and Jaccard used in both, ITT and PP population. As example, in the ITT population the proportion of responders, using the Dice algorithm, was 31.6% in Amoxicillin plus Enterogermina® treated patients and 28.6% in Amoxicillin treated patients. (Odds ratio was 1.15 (95% CI: 0.30; 4.47, p-value 0.836). Median Similarity Coefficient (MSC) for the ITT population obtained with the Dice algorithm was very similar between treatments (74.4% and 74.6% in Amoxicillin plus Enterogermina® and Amoxicillin group respectively), with a moderate Inter Quartile Range (IQR) (13.6% and 18.8%).

	Mean similarity coefficient was also very similar between treatments (74.4% and 72.4% respectively in Amoxicillin plus Enterogermina® and Amoxicillin group). The mean difference between the two groups was 2.03% (95% CI: -5.31; 9.38) and not statistically significant (p-value 0.578). Using Dice and Jaccard algorithms, a relationship between the proportion of responders and age was observed. The proportion of responders was higher in the patients aged more than 2 years than in patients aged under 2 years. In particular, according to the Dice algorithm the proportion of responders was 45.8% (11/24) in the patients aged more than 2 years and 6.3% (1/16) in patients aged under 2 years, the odds ratio was 12.69 (95% CI: 1.44; 112.02, p-value 0.007). An ANOVA analysis performed on subgroups identified by each age class revealed, that for younger patients (≤ 2 years) the mean Dice similarity coefficient (%) on ITT set was greater in the Amoxicillin plus Enterogermina® group (71.1%) than Amoxicillin group (61.5%). The mean difference between the two groups was 9.66% (95% CI: 2.48; 16.84) and statistically significant (p-value 0.016). The results for the mean Jaccard similarity coefficient were similar. <u>Secondary efficacy variable(s)</u> No change in body weight was observed in any group at the end of treatment [ITT population) (p-value 0.167). Further secondary end-point was the change of abdominal symptoms such as discomfort, bloating, straining, stool frequency and consistency, reported daily on patient's diary in terms of a point rating scale or presence/absence. No statistically significant change over study treatment nor difference between treatment groups was found for the change of abdominal symptoms comparing day one versus last not missing value. Only for abdominal bloating the difference in the Amoxicillin group was statistically but not clinically significant (p-value 0.037). It is noticeable that concerning diarrhea no differences were observed between groups in term of both frequency and consistency at the end of treatment . Amelioration of this parameter over the time for both groups was observed with no statistical difference beetween groups. No ITT patient reported GI symptoms over the study period. A relationship between the number of *Bacillus clausii* spores and similarity coefficient was observed. The Spearman coefficient result was 0.70 (p-value 0.011).
Safety results:	The number of patients reporting TEAEs over the observation period was 12 (41.4%) in the Amoxicillin plus Enterogermina® group and 14 (53.8%) in the Amoxicillin group. The relative risk was 0.768 (95% CI: 0.439 – 1.346). Only 1 serious TEAE was reported for a patient in the Amoxicillin plus Enterogermina® group. The proportion of patients who experienced an TEAE leading to discontinuation was higher in the Amoxicillin group (7.7%) than in the Amoxicillin plus Enterogermina® group (3.4%). The relative risk was 0.448 (95% CI: 0.043 – 4.660). No treatment-related TEAEs or deaths were observed in the study. The most common TEAEs were symptoms commonly observed during the underlying condition: pyrexia (Amoxicillin plus Enterogermina® 24.1% vs. Amoxicillin 23.1%), ear pain (Amoxicillin plus Enterogermina® 0% vs. Amoxicillin 11.5%), cough (Amoxicillin plus Enterogermina® 0% vs. Amoxicillin 11.5%) and vomiting (Amoxicillin plus Enterogermina® 10.3% vs. Amoxicillin 3.8%). No relevant difference in changes of body temperature was observed between the two treatment groups. In conclusion, the two treatments were well tolerated and no clinically meaningful safety issues were raised during the study.
Date of report:	29-Sep-2008