Original Investigation / *Özgün Araştırma*

DOI: 10.5578/ced.66936 • *J Pediatr Inf 2017; 11(4): e154-e158*

Susceptibility of *Salmonella* Isolates to Commonly Used Antimicrobial Drugs at a Tertiary Care Hospital in Izmir

İzmir'de Bir Üçüncü Basamak Hastanede İzole Edilen *Salmonella* Türlerinin Sıkça Kullanılan Antimikrobiyal İlaçlara Olan Duyarlılıkları

Mine Düzgöl[1], Ahu Kara[1], İlknur Çağlar[1], Nuri Bayram[1], Yüce Ayhan[2], Gamze Gülfidan[2], Hurşit Apa[3], İlker Devrim[1]

[1] *Clinic of Pediatric Infectious Diseases, Dr. Behcet Uz Pediatric Diseases Training and Research Hospital, Izmir, Turkey*
[2] *Clinic of Medical Microbiology, Dr. Behcet Uz Pediatric Infectious Diseases Training and Research Hospital, Izmir, Turkey*
[3] *Clinic of Pediatric Health and Diseases, Dr. Behcet Uz Pediatric Infectious Diseases Training and Research Hospital, Izmir, Turkey*

Abstract

Objective: Aim of this study was to evaluate antibiotic resistance of salmonella strains isolated from various samples in a tertiary pediatric care center in Turkey.

Material and Methods: In the current study, all *Salmonella* strains isolates from clinical specimens of pediatric patients were evaluated in the terms of antibiotic susceptibility tests. Between April 2007 and October 2014, clinical characteristics and treatment outcomes of patients with salmonella infection, as well as serotype distribution and antibiotic resistance of salmonella strains were evaluated retrospectively by using medical database of Dr. Behçet Uz Children's Hospital.

Results: A total of 76 *Salmonella* strains were examined. For 52 (68.4%) patients' stool cultures were performed, and blood cultures were positive for 18 (23.7%) patients from whom specimens were drawn. Other specimens include 2 (2.6%) samples from central venous catheter, 3 samples (3.9%) from urine and 1 (1.3%) in culture of purulent material obtained from peritoneal fluid. The most isolated serotypes were *S. enterica* serovar Typhi (7.9%, n= 6) and *S. enterica* serovar Arizonae (6.5%, n= 5), the other serotypes were detected as *S. enterica* serovar Enteritidis (3.9%, n= 3), *S. paratyphi* A (2.5%, n= 2) and *S. paratyphi* B (1.3%, n= 1). Nine (15%) isolates in typhoid fever were showed no resistance to trimethoprim-sulfamethoxazole and ciprofloxacin but 22% and 11% of the isolates were resistant to ampicillin and ceftriaxone. Non-typhoid *Salmonella* isolates resistance to ampicillin was 33%, trimethoprim-sulfamethoxazole resistance was 12%, ceftriaxone resistance was 14%, and 3% isolates was detected in ciprofloxacin resistance. We emphasized

Özet

Giriş: Bu çalışmanın amacı, Türkiye'de üçüncü basamak bir pediatrik bakım merkezindeki çeşitli örneklerden izole edilen *Salmonella* suşlarının antibiyotik direncini değerlendirmektir.

Gereç ve Yöntemler: Çocukların klinik örneklerinden elde edilen bütün *Salmonella* izolatları çalışmaya dahil edildi ve farklı antibiyotikler açısından duyarlılık testleri çalışıldı. Nisan 2007 ve Ekim 2014 arasında, enfeksiyon etkeni olarak saptanan *Salmonella* suşlarının serotip dağılımı ve antibiyotik direncinin yanı sıra klinik özellikleri ve tedavi sonuçları, hastane tıbbi veritabanı kullanılarak, geriye dönük olarak değerlendirildi.

Bulgular: Toplam 76 *Salmonella* suşu incelendi. Bunların 52 (%68.4)'si gaita kültürü, 18 (%23.7)'i kan kültürü, 3 (%4)'ü idrar kültürü, 2 (%2.6)'si santral venöz kateter ve 1 (%1.3)'i periton sıvısından alınan pürülan materyal kültüründe üreyen suşlardı. Kültürde 17 (%22) hastada *Salmonella* serotipinin belirlendiği görüldü. En sık izole edilen serotipler, *S. enterica* serovar Typhi (%7.9, n= 6), *S. enterica* serovar Arizonae (%6.5, n= 5) olup, *S. enterica* serovar Enteritidis (%3.9, n= 3), *S. paratyphi* A (%2.5, n= 2) ve *S. paratyphi* B (%1.3, n= 1) diğer saptanan serotiplerdir. Tifoid ateşi olan 9 hastada trimetoprim-sülfametoksazol ve siprofloksasine direnç saptanmazken, ampisilin direncinin %22, seftriakson direncininise %11 oranında olduğu görüldü. Tifo dışı *Salmonella* suşlarında ampisiline %33, trimetoprim-sülfametoksazole %12, seftriaksona %14 ve siprofloksasine %3 oranında direnç saptandı. Tifo dışı *Salmonella* izolatları arasında çoklu ilaç direnci insidansının ise %3 (sadece iki hasta) gibi düşük bir oranda olduğu tespit edildi.

Correspondence Address / Yazışma Adresi

Mine Düzgöl
Dr. Behçet Uz Çocuk Hastalıkları Eğitim ve Araştırma Hastanesi, Çocuk Enfeksiyon Hastalıkları Kliniği, İzmir-Türkiye

E-mail: mineduzgol@gmail.com

Conclusion: In conclusion there was still low MDR in both typhoid and non-typhoid Salmonella serotypes. Detection of serotype and follow-up of antibiotic resistance would be useful for the proper antimicrobial treatment of serious infections caused by *Salmonella* strains.

Keywords: Antimicrobial drugs, salmonella, susceptibility

Sonuç: Sonuç olarak, tifoid ve tifo dışı *Salmonella* serotiplerinin her ikisinde de çoklu ilaç direnci insidansı hala düşük orandadır. Serotip belirlenmesi ve antibiyotik direnci takibi, *Salmonella* suşlarının neden olduğu ciddi enfeksiyonların uygun antimikrobiyal ilaç ile tedavi edilmesi açısından yararlı olacaktır.

Anahtar Kelimeler: Antimikrobiyal ilaçlar, salmonella, duyarlılık

Introduction

It is estimated that ten millions of *Salmonella* infections occur worldwide every year and result in more than hundred thousand deaths (1). The widespread species involve more than 2600 different serotypes that can be divided mainly into typhoid and non-typhoid *Salmonella* serotypes. Nevertheless their genetic similarity, these two groups result in different types of diseases and various immune responses in human (2).

Drugs such as aminoglycosides, polymyxins, tetracyclines, and cephalosporins were reported to have a poor clinical response, despite apparent in vitro susceptibility. Various antibiotics were used in salmonella infections, but increasing number of multidrug resistant (MDR) *Salmonella* serotypes which was identified by the Centers for Disease Control and Prevention (CDC) National Antimicrobial Resistance Monitoring System (3). In '90s, for the treatment of infections, the physicians moved out the use of first-line antibiotics such as chloramphenicol, ampicillin, and cotrimoxazole due to multidrug resistance. Since then, fluoroquinolones have been used for the primary treatment for salmonellosis in adult patients. However, the concerns about toxicity of fluoroquinolones in children, their use in these MDR *Salmonella* infections have been limited. In areas with MDR isolates, third generation cephalosporins might be recommended as a choice in children. Azithromycin also may be used as a drug with activity against both nalidixic acid resistant and MDR serotypes (4,5).

Data on the resistance patterns of *Salmonella* serotypes and also treatment strategies are mainly based on adult studies. Currently, there are limited reports concerning the MDR status in *Salmonella* infections in pediatric patients. Therefore, the current study aimed to identify the resistance patterns in isolated *Salmonella* serotypes and also treatment opportunities in children.

Materials and Methods

The present study was carried out in clinical samples of children who were hospitalized at Dr. Behçet Uz Children's Hospital from April 2007 to October 2014. All children with infection disease due to *Salmonella* serotypes isolated from blood, stool, urine, peritoneal fluids, and other sterile sites were evaluated. Data of the patients was collected from medical records including information on demographic characteristics (age, gender), underlying diseases or co-morbidities, and antimicrobial susceptibility patterns of the isolated serotypes.

Identifications of the samples were performed using the usual techniques, confirmed by the API 20E system (bioMérieux, France) and serotyped with respect to somatic (O) and flagellar (H) antigens using commercial antisera (Refik Saydam Hygiene Center, Turkey). As all isolates were identical in serotyping and had the same antimicrobial resistance profile.

Antibiotic susceptibility tests: Susceptibility patterns of all samples and their transconjugants to ampicillin, ciprofloxacin, ceftriaxone, and trimethoprim-sulfamethoxazole were estimated by Clinical and Laboratory Standards Institute (CLSI) disk diffusion method (6). Minimal inhibitory concentration (MIC) was evaluated by CLSI microdilution method (7). *Escherichia coli* ATCC 25922 and *Pseudomanas aeruginosa* ATCC 27853 were used as control strains.

Multidrug resistant *Salmonella* serotypes were defined as detected strains of *Salmonella* which were resistant to the first-line recommended drugs for treatment such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole.

Statistical analysis was analyzed using SPSS software version 20.0 (IBM Corp., Somers, NY). Categorical variables were analyzed using a chisquare or Fisher exact test. Continuous variables were analyzed using Student's t test or the Mann-Whitney U test. A P value of < 0.05 denoted statistically significance.

The study was approved by the local ethics committee of Dr. Behçet Uz Children's Hospital.

Results

Salmonella serotypes were isolated in 76 patients with the median age of 59 months ranging from 1 month to 170 months. Twenty patients (26.3%) were under 24 months of age and 39 (51.3%) were younger than 5 years of age. Among 76 patients, 41 were male (54%) and 34 were female (46.0%).

Of isolated 76 samples; 52 (68.4%) were isolated in stool, 18 (23.7%) in blood, 3 (3.9%) in urine, 2 (2.6%) in central venous catheters, and 1 (1.3%) in peritoneal fluid sample (Figure 1). *Salmonella enterica* serovar Typhi (7.9%, n= 6) and *S. enterica* sero-

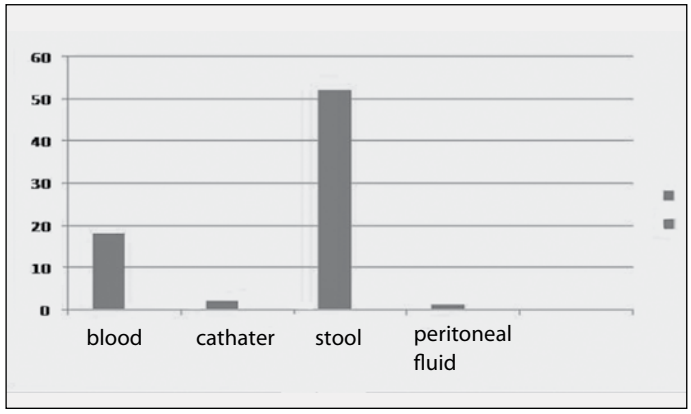

Figure 1. The number of *Salmonella* serotypes isolated, according to their sources.

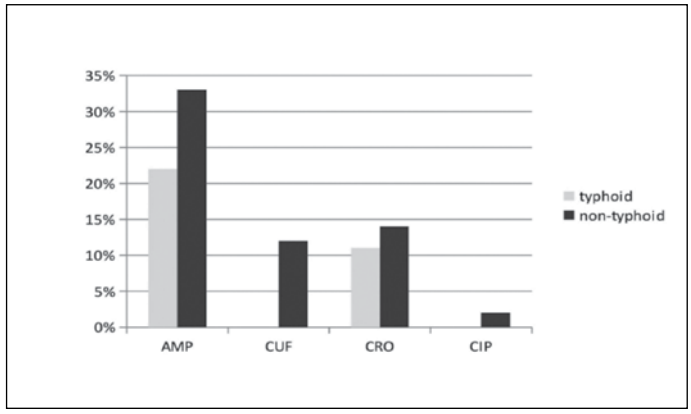

Figure 2. The percentage antibiotic resistance of typhoid and non-typhoid *Salmonella* serotypes

AMP: Ampicilin, CUF: Trimethoprim-sulfametoxazole, CRO: Ceftriaxon, CIP: Ciprofloxacin.

var Arizonae (6.5%, n= 5) were the most common serotypes. The other detected serotypes were *S. enterica* serovar Enteritidis (3.9% ,n= 3), *S. paratyphi* A (2.5%, n= 2) and *S. paratyphi* B (1.3%, n= 1).

Salmonella serotypes were detected in blood sample from one patient with IL-12 deficiency, one patient with cerebral palsy, 3 patients with acute lymphoblastic leukemia (ALL), one patient with T- cell deficiency, 2 patients with hemophagocytic lymphohistiocytosis (HLH), one patient with cytomegalovirus hepatitis and stool sample from 2 patient with cerebral palsy, 4 patient with ALL. The patient with HLH exhibit severe hypoxia and hypercarbia and died of respiratory failure.

Enteric fever was diagnosed in 9 patients. Of these patients; *S. enterica* serovar Typhi was isolated from 6 patients, the other isolates were *S. paratyphi* A from 2 patients and *S. paratyphi* B from one patient. Seven patients diagnosed with typhoid fever (77%) showed sensitivity to all antibiotic disks [ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole (TMP-SMX), and ceftriaxone]. Nine (15%) isolates in typhoid fever were showed no resistance to TMP-SMX and ciprofloxacin, but 22 % and 11 % of the isolates were resistant to ampicillin and ceftriaxone (Figure 2).

Thirty-eight non typhoid *Salmonella* isolates (56%) showed sensitivity to all antibiotic disks (ampicillin, ciprofloxacin, ceftriaxone, and TMP-SMX).

In non-typhoid *Salmonella* isolates resistance to ampicillin, trimethoprim-sulfamethoxazole, ceftriaxon, ciprofloxacin was detected in 33%, 12%, 14%, and 3%, respectively (Figure 2).

In all immuncompromised children, only non-typhoid *Salmonella* isolates were detected (n= 11). One non-typhoid *Salmonella* isolated from patient with IL-12 deficiency showed antibiotic resistance to ampicillin, ceftriaxone, TMP-SMX but was sensitive to ciprofloxacin. One patient with ALL showed antibiotic resistance to ampicillin, ciprofloxacin, TMP-SMX but was sensitive to ceftriaxone; the other non-typhoid *Salmonella* isolates in immunocompromised children showed sensitivity to all antibiotics. Nine typhoid *Salmonella* isolates and 56 non-typhoid salmonella isolates were detected from immunocompetent children.

Discussion

In the current study, *S. enterica* serovar Typhi and *S. enterica* serovar Arizonae were the most isolated serotypes. It was established that incidence of MDR strains were lower among non-typhoid salmonella isolates compared to the typhoid isolates.

Salmonella infections are major public health problem in Turkey. Although *S. enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium are detected as the most common serotypes in Turkey, and the incidence of *S. enterica* serovar Typhi and *S. paratyphi* B is gradually increasing (8,9).

Salmonella serotype distribution may vary according to different geographic regions and years. A retrospective study in European the most frequently isolated *Salmonella* strains were reported as *S. enterica* serovar Enteritidis (59%), followed by *S. enterica* serovar Typhimurium (4.7%), *S. enterica* serovar Virchow (2.6%), *S. enterica* serovar Newport (1.8%) (10). In this study, the most isolated serotypes were *S. enterica* serovar Typhi and *S. enterica* serovar Arizonae. Nine patients were diagnosed as enteric fever and the most common isolated serotypes in patients with enteric fever was *S. enterica* serovar Typhi, suggesting the other studies (10).

Empirical antimicrobial treatment for invasive salmonellosis in children includes a third generation cephalosporins. Once susceptibilities are available, narrower-spectrum therapy includes ampicillin, amoxicillin, as well as broader-spectrum agents such as chloramphenicol, TMP-SMX, or a fluoroquinolone (11).

For enteric fever caused by *S. enterica* serovar Typhi infection, initial empiric therapy with ceftriaxone is recommended due to widespread resistance. Duration of therapy should be at least 14 days (12). *Salmonella enterica* serovar Typhi is especially showing resistance to ciprofloxacin in United States of America (USA). Resistance or partial resistance to ciprofloxacin was 67% of all *S. enterica* serovar Typhi in USA (three year average from 2009 to 2011). In USA drug resistance to *S. enterica* serovar Typhi has jumped significantly from about 20% in 1999 to more than 70% in 2011 (1). *Salmonella enterica* serovar Typhi and *S. paratyphi* B are usually determined as phenotypically by antimicrobial resistance patterns and serotype in studies in Turkey. In Turkey, the most studies were shown that *S. enterica* serovar Typhi and *S. paratyphi* B isolates are susceptible to all tested antimicrobials (13). In this study, we detected only one *S. enterica* serovar Typhi isolate showed antibiotic resistance to ampicillin and ceftriaxone but sensitivity to TMP-SMX and ciprofloxacin. The other isolates (83%) were showed high sensitivity to all antibiotic disks (ampicillin, ciprofloxacin, ceftriaxone, and trimethoprim-sulfamethoxazole).

As many as 40% of non-typhoid *Salmonella* isolates in the USA are multidrug resistant (MDR), with increasing resistance to all *Salmonella* strains worldwide (14). In particular parts of the world (ie, India, Pakistan, Egypt), MDR strains of non-typhoid *Salmonella* isolates were reported. Decreased ciprofloxacin susceptibility and ceftriaxone resistance has been reported in developing countries (15-17). Multidrug resistant *Salmonella* isolates are particularly common in the Indian subcontinent, Southeast Asia, and Africa, and resistance to ciprofloxacin is increasing (18). In Turkey, MDR among *S. enterica* serovar Typhimurium strains have been firstly detected in 1970 (19). In 2001, Extended-spectrum beta-lactamases production of salmonella isolates from Turkey was determined in 15% (20). A recent study from Turkey determined high incidence of MDR among *S. enterica* serovar Typhimurium, at a rate of 76 (13,21).

Fluoroquinolone drugs have used safely in all age groups, associated with lower rates of stool carriage than traditional first-line drugs and rapidly effective. The three main difficulties concerning the use of fluoroquinolones are toxic effects on children, the price, and resistance. Because of the high level of resistance in some regions of the world, the World Health Organization recommendations for second-line therapy options include third generation cephalosporins and azithromycin (22). In this thirty-eight non-typhoid *Salmonella* isolates (56%) showed sensitivity to all antibiotics. We emphasized that the low incidence of MDR among non-typhoid *Salmonella* isolates (only two patients) at a rate of 3%.

The risk of invasive salmonellosis and non-typhoid salmonella infections are increased in the patients with immunosuppression, especially impaired cell-mediated immunity, lymphoproliferative diseases and in patients with IL-12 deficiency (23). In all immunocompromised children, non-typhoid salmonella isolates were detected (n= 11). One non typhoid salmonella isolate from patient with IL-12 deficiency showed antibiotic resistance to ampicillin, ceftriaxone, TMP-SMX but sensitivity to ciprofloxacin, one patient with ALL showed antibiotic resistance to ampicillin, ciprofloxacin, TMP-SMX but sensitivity to ceftriaxone; the other non-typhoid *Salmonella* isolates in immunocompromised children showed sensitivity to all antibiotics.

The present study has some limitations, firstly due to its retrospective study design. Secondly, only the microbiologic culture results were interpreted, the clinic status or outcomes of the patients were not analyzed.

In conclusion we reported that there was very low MDR in both typhoid and non-typhoid *Salmonella* serotypes. It is important to detection of serotype and follow-up of antibiotic resistance would be useful for the treatment of serious infections caused by salmonella strains.

Ethics Committe Approval: Ethics committee approval was received for this study from Dr. Behcet Uz Pediatric Diseases Training and Research Hospital local Ethics Committee.

Informed Consent: Written informed consent was not received due to this study include isolated bacterial species.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – MD, İD; Design - MD, İD; Supervision – MD, İD; Data Collection and/or Processing - MD, AK, İÇ, NB, YA ,GG, HA, İD; Analysis and/or Interpretation - MD, İD; Literature Review - MD, İD; Writing – MD, NB; Critical Review – MD, İD.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. *CDC. Typhoid fever. http://www.cdc.gov/nczved/divisions/dfbmd/diseases/typhoid*
2. *Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. Front Microbiol 2014;5:391.*
3. *Goldrick BA. Foodborne diseases. Am J Nurs 2003;103:105-6.*
4. *Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis 2001;32:263-9.*
5. *Arjyal A, Pandit A. Treatment of enteric fever. J Infect Dev Ctries 2008;2:426-30.*
6. *The Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests, 9th Informational Supplement, 2006.*

7. *The Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically, 7th Informational Supplement, 2006.*
8. *Toreci K, Ang O. Türkiye'de saptanmış olan Salmonella serovarların ve Salmonellozların genel değerlendirilmesi. Türk Mikrobiyol Cem Derg 1991;21:1-18.*
9. *Erdem B, Ercis S, Hascelik G, et al. Antimicrobial resistance patterns and serotype distribution among Salmonella enterica strains in Turkey, 2000-2002. Eur J Clin Microbiol Infect Dis 2005;24:220-5.*
10. *Matheson N, Kingsley RA, Sturgess K, et al. Ten years experience of Salmonella infections in Cambridge, UK. J Infect 2010;60:21-5.*
11. *Kimberlin DW, Brady MT, Jackson MA, et al. Salmonella infections. Red Book: Report of the Committee on Infectious Diseases. 29th ed. American Academy of Pediatrics 2012;635-41.*
12. *Typhoid fever. Protocol for Diagnosis and Management of Typhoid Fever in Children. IAP Infectious Diseases Chapter Publication, 2007.*
13. *Erdem B, Ercis S, Hascelik G, et al. Antimicrobial resistance of Salmonella enterica group C strains isolated from humans in Turkey, 2000-2002. Int J Antimicrob Agents 2005;26:33-7.*
14. *Long A, Pickering, L, Prober C. Salmonella Species. Principles and Practices of Pediatric Infectious Diseases Elsevier, 2012.*
15. *Gordon MA, Graham SM, Walsh AL, et al. Epidemics of invasive Salmonella enterica serovar enteritidis and S. enterica Serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. Clin Infect Dis 2008;46:963-9.*
16. *Egorova S, Timinouni M, Demartin M, et al. Ceftriaxone-resistant salmonella enterica serotype Newport, France. Emerg Infect Dis 2008;14:954-7.*
17. *Crump JA, Medalla FM, Joyce KW, et al. Emerging Infections Program NARMS Working Group. Antimicrobial resistance among invasive nontyphoidal Salmonella enterica isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. Antimicrob Agents Chemother 2011;55:1148-54.*
18. *Chuang CH, Su LH, Perera J, et al. Surveillance of antimicrobial resistance of Salmonella enterica serotype Typhi in seven Asian countries. Epidemiol Infect 2009;137:266-9.*
19. *Otkun M, Erdem B, Akata F, et al. Antibiotic resistance patterns and plasmid profiles of Salmonella typhimurium isolates in Turkey. Eur J Clin Microbiol Infect Dis 2001;20:206-9.*
20. *Kiliç D, Tulek N, Tuncer G, et al. Antimicrobial susceptibilities and ESBL production rates of Salmonella and Shigella strains in Turkey. Clin Microbiol Infect 2001;7:341-2.*
21. *Ağın H, Ayhan FY, Gülay Z, et al. The evaluation of clusters of hospital infections due to multidrug-resistant Salmonella enterica serovar typhimurium in the neonatal unit: a two-year experience. Turk J Pediatr 2011;53:517-21.*
22. *Recommendations for management of common childhood conditions: newborn conditions, dysentery, pneumonia, oxygen use and delivery, common causes of fever, severe acute malnutrition and supportive care. World Health Organization, 2012.*
23. *De Jong R, Altare F, Haagen IA, et al. Severe mycobacterial and Salmonella infections in interleukin-12 receptor-deficient patients. Science 1998;280:1435-8.*